I U C L I D

Data Set

Existing Chemical	:	ID: 40601-76-1
CAS No.	:	40601-76-1
EINECS Name	:	1,3,5-Tris[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-xylyl]methyl]1,3,5-triazine-2,4,6(1H,3H,5H)-trione
Producer related part		
Company	:	Cytec Industries Inc.
Creation date	:	05.05.2003
Substance related part		
Company	:	Cytec Industries Inc.
Creation date	:	05.05.2003
Status	:	
Memo	:	
Printing date	:	01.06.2005
Revision date	:	01.06.2005
Date of last update	:	01.06.2005
Number of pages	:	62
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	Cytec Industries Inc.
Contact person	:	
Date	:	
Street	:	5 Garret Mountain Plaza
Town	:	07424 West Patterson, NJ
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

Reliability : (1) valid without restriction
12.04.2005

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	None assigned
Smiles Code	:	O=C3(N(Cc1(c(C)c(O)c(C(C)(C)C)cc1C))C(=O)N(Cc2(c(C)c(O)c(C(C)(C)C)cc2C))C(=O)N3Cc4(c(C)c(O)c(C(C)(C)C)cc4C))
Molecular formula	:	C42 H57 N3 O6
Molecular weight	:	699.94
Petrol class	:	

21.05.2003

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	
Substance type	:	organic
Physical status	:	solid
Purity	:	= 93.7 - 98.9 g/kg
Colour	:	off-white powder
Odour	:	none

14.04.2005 (3)

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1,3,5-tris((4-tert-butyl-3-hydroxy-2,6-xylyl)methyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione

21.05.2003

1,3,5-tris(2,6-dimethyl-3-hydroxy-4-tert-butyl) isocyanurate

21.05.2003

1,3,5-tris(4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl)methyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione

18.03.2004

1,3,5-tris(4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione

12.04.2005

1,3,5-tris(4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl)-s-triazine-2,4,6-(1H,3H,5H)-trione

12.04.2005

Cyanox (TM) 1790 Antioxidant

12.04.2005

tris(4-t-butyl-3-hydroxy-2,6-dimethylbenzyl)-s-triazine-2,4,6-(1H,3H,5H)-trione

12.04.2005

tris(4-tert-butyl-2,6-dimethyl-3-hydroxybenzyl)isocyanurate

12.04.2005

tris(4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl)isocyanurate

12.04.2005

1.3 IMPURITIES

1.4 ADDITIVES

Remark : No additives
Source : Cytec Industries Inc.
12.04.2005

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use	:	industrial
Category	:	Polymers industry
Remark	:	Tris(4-t-butyl-3-hydroxy-2,6-dimethylbenzyl)-s-triazine-2,4,6(1H,3H,5H)-trione is an effective antioxidant for a variety of polymer systems. This substance provides superior polymer stabilization with minimal color contribution and low volatility. The substance may be used in food packaging materials and polystyrene and rubber modified polystyrene as an antioxidant. As such, it is cleared by the FDA under 21 CFR (Code of Federal Regulations) Section 178.2010. The material is also used as an antioxidant in polyethylene polymer, at the 0.05-0.10% level. The substance is melt-soluble in the polymer, and has demonstrated limited potential for migration from the polymer (Kawamura et al., 1997). The material is also used as an antioxidant in fiber (such as Spandex® PUR fiber) at the 0.5-1.5% level.

12.04.2005

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	= 159 - 162 °C
Sublimation	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions Experimental details were not provided.
Flag	:	Critical study for SIDS endpoint

19.05.2003 (3)

Value	:	ca. 349.8 °C
Sublimation	:	
Method	:	other: calculated
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	A CAS No. of 40601-76-1 was inputted. Value given is the average of the Joback, Gold and Ogle methods.
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.

14.04.2005 (11)

2.2 BOILING POINT

Value	:	ca. 926 °C at 1013 hPa
Decomposition	:	
Method	:	other: calculated
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Inputs to the program are CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C.
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.
Flag	:	Critical study for SIDS endpoint

12.04.2005 (11)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	ca. .000013 hPa at 25 °C
Decomposition	:	
Method	:	other (calculated)
Year	:	2003

GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Inputs to the program are CAS No. 40601-76-1 and a measured melting point of 160.5 degrees C.
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.
Flag	:	Critical study for SIDS endpoint

19.05.2003 (11)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= 15.281 at °C
pH value	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The CAS No. 40601-76-1 was inputted into the program.
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.
Flag	:	Critical study for SIDS endpoint

12.04.2005 (10)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	< .00002 g/l at 20 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	OECD Guide-line 105
Year	:	2004
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4

Result : The preliminary estimate of water solubility was <1.5 x E-3 g/l. In the definitive test the mean concentrations in both Determination A and Determination B were <2.0 x E-5 g/l.

The linearity of the detector response with respect to concentration was assessed over the nominal concentration range of 1.0 to 0.01 mg/l. This was satisfactory with a correlation coefficient of 0.999 being obtained.

With the exception of two analytical samples, no detectable quantities of test material were present in any sample solutions on analysis. Therefore, a limit value which incorporated the trace concentrations in these two samples has been reported as the definitive result. The overall limit value of less than 2.0 x E-5 g/l of solution was calculated from the nominal concentration of the lowest validation linearity standard shown to be detectable (0.01 mg/l) corrected for dilution factor (2).

Test condition	:	The determination was carried out using the column elution method with a circulating pump (Method 105 of the OECD Guidelines of July 27, 1995 and Method 830.7840 of the OPPTS Guidelines). A preliminary test was run by diluting an aliquot of the test material (0.1010 g) to 100 ml with double glass-distilled water. After shaking at 30 degrees C for 4 1/4 hours and standing at 20 degrees C for 17 ½ hours, the solution was centrifuged and analyzed. In the definitive test, an aliquot (0.1023 g) of test material and glass beads (approximately 1.0 g) were added to a round bottom flask and the test material dissolved in acetonitrile (30 ml). The solvent was then removed using a rotary evaporator. The elution apparatus was set up consisting of a glass micro-column fitted with a plug of glass wool, connected to a re-circulating pump and a reservoir capable of holding approximately 500 ml of water. The circulating water was maintained at 20.0 +/- 0.5 degrees C by means of a water bath fitted with a cooling coil. The whole system was flushed overnight with distilled water, which was then discarded. The reservoir was refilled with fresh glass double-distilled water and the coated glass beads loaded into the micro-column. After allowing the coated beads to soak for approximately 2 hours, the recirculating pump was switched on and the first 25 ml of the eluate discarded. Aliquots of sample solution were taken from the column at intervals of at least ten bed volumes of eluate and centrifuged at 13,500 rpm for 20 minutes. The procedure was carried out in duplicate (A and B). The concentration of test material in the sample solutions was determined by high performance liquid chromatography. The mean peak area of each standard was corrected to a nominal concentration of 0.5 mg/l and the mean value taken. The sample solution concentration (g/l) was calculated using the following equation:C(spl) = [P(spl/P(std)] x C(std) x D x 0.001, where: C(spl) = sample concentration (g/l); P(spl) = mean peak area of sample solution; P(std) = mean peak area of standard solution, corrected to nominal standard concentration; C(std) = nominal standard concentration (15 g/l) and D = sample dilution factor (2).
Test substance	:	The lot number of the test substance was PA 4040608.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

12.04.2005 (22)

Solubility in	:	Water
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: calculated
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The CAS No. 40601-76-1 was inputted into the program.
Result	:	2.425 E-11 mg/l at 25 °C
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.

14.04.2005 (12)

Solubility in	:	Water

Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Remark	:	Negligible solubility in water.
Reliability	:	(4) not assignable Experimental details were not provided.

14.04.2005 (3)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	= .0000000000908947 cm³/(molecule*sec)
Degradation	:	= 50 % after 1.4 hour(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The CAS No. 40601-76-1 was inputted into the program. Atmospheric photodegradation is not expected to be a significant route of elimination, since the substance has very limited volatility.
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.
Flag	:	Critical study for SIDS endpoint

14.04.2005 (5)

3.1.2 STABILITY IN WATER

Type	:	
t1/2 pH4	:	at °C
t1/2 pH7	:	ca. 1 year at 20 °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The CAS No. 40601-76-1 was inputted into the program. EPIWIN Hydrowin estimates that the rate of hydrolysis in neutral water at ambient temperatures will be extremely slow, with a half life of > 1 year. This estimate is based on the presence of urea functional groups in the molecule.
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.

14.04.2005 (9)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	soil - air
Air	:	0 % (Fugacity Model Level I)
Water	:	1.28 % (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	67.1 % (Fugacity Model Level II/III)
Soil	:	31.6 % (Fugacity Model Level II/III)
Method	:	other
Year	:	2003
Remark	:	Inputs to the model were the CAS No. 40601-76-1, a measured melting point of 160.5 degrees C and a measured water solubility of 0.02 mg/l. Emission rates are assumed to be 0 kg/hr to air, 1000 kg/hr to water, 1000 kg/hr to soil, and 0 kg/hr to sediment.
Result	:	Half-lives in various media are air: 2.825 hours; water; 3600 hours; soil: 3600 hours; and sediment: 1.44 E+4 hours. The Henry's Law Constant [calculated by EPIWIN Henry (v3.10)] is 1.7E-27 atm-m3/mol (bond est.). The soil-sediment coefficient Koc [calculated by EPIWIN PCKOC (v1.66)] is Koc = 1E+10.
Reliability	:	(2) valid with restrictions Data were obtained by EPIWIN modeling.
Flag	:	Critical study for SIDS endpoint

14.04.2005 (8)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other bacteria: secondary effluent from wastewater treatment plant
Contact time	:	28 day(s)
Degradation	:	= 25.9 (±) % after 28 day(s)
Result	:	other: not readily biodegradable
Kinetic of testsubst.	:	5 day(s) = 2.3 % 10 day(s) = 5.4 % 14 day(s) = 9.3 % 20 day(s) = 17.2 % 28 day(s) = 24.7 %
Control substance	:	Benzoic acid, sodium salt
Kinetic	:	% %
Deg. product	:	not measured
Method	:	OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Remark : The authors stated that "the test substance was not toxic to the inoculum since biodegradation occurred in a mixed solution containing test and reference materials". However, it is clear that the test material was toxic to the inoculum, since the amount of biodegradation occurring in the flask containing both the reference and test material was less than that of the reference material alone.

Result : The cumulative biodegradation rates (in %) for the two concentrations of test material at 3, 5, 7, 10, 14, 17, 20, 24, 28 and 29 days were 1.0 and 1.0, 2.1 and 2.4, 4.5 and 4.57, 4.98 and 5.83, 8.28 and 10.4, 13.3 and 15.4, 16.3 and 18.1, 17.4 and 21.8, 21.7 and 27.6 and 23.2 and 28.5, respectively. At the same time intervals, values (in %) for inoculum controls were 12.9 and 12.7, 15.1 and 15.4, 17.2 and 14.4, 22.0 and 22.7, 25.6 and 23.5, 20.7 and 21.2, 24.4 and 23.4, 25.5 and 24.5, 23.3 and 23.4 and 20.1 and 19.6, respectively. The cumulative biodegradation rate for the reference material at 3, 5, 7, 10, 14, 17, 20, 24, 28 and 29 days was 19.0, 37.0, 54.0, 62.4, 68.1, 71.6, 73.6, 74.4, 76.4 and 77.1, respectively. The cumulative biodegradation rate for the reference plus test material at 3, 5, 7, 10, 14, 17, 20, 24, 28 and 29 days was 9.5, 18.4, 26.9, 32.0, 35.4, 37.3, 38.3, 38.8, 39.6 and 40.5, respectively.

Since a >= 60% biodegradation rate was not achieved within 28 days, the material was not deemed readily biodegradable. The test was valid, since the total amount of CO2 evolution in the inoculum blank at the end of the test was within the acceptable range for a valid test, and the cumulative biodegradation of the reference material (68.1% on day 14) was > = 60% by day 14. The difference in CO2 evolution between extreme duplicate values of test material at the end of the test (18.6%) also met the requirements of the guideline (< 20%).

Test condition : Water used for the preparation of test media was collected from a water purification system using both ion exchange and carbon filtration treatment. The test water had a total organic carbon (TOC) content < 1 mg/l. TOC analysis was done using a DC-80 TOC analyzer system. This water was used to make a defined mineral medium containing phosphate buffer, ferric chloride (final concentration 0.25 mg/l), magnesium sulfate (final concentration 22.5 mg/l) and calcium chloride (final concentration 27.5 mg/l). The test material was not soluble enough in water to make a stock solution, so the material was weighed into weigh boats and added dry to test bottles. The weigh boats were washed with mineral medium to remove as much material from them as possible. The carbon content of the test material was analyzed with an elemental analyzer. It contained the equivalent carbon concentration of 71.9%. A stock solution of the reference material (1000 mg ai/l 99.6% pure sodium benzoate) was prepared on the day of the test which had an equivalent carbon concentration of 58.3%.

A total of six 4-liter bottles were prepared. On day minus one, 100 ml of inoculum and 2400 ml of mineral medium were added to each flask. The inoculum was the glass wool filtrate from 2 liters of secondary effluent collected on May 30, 2002 from the Loxahatchee River Environmental Control District Wastewater Treatment Plant in Jupiter, Florida. The contents of each bottle were purged with CO2-free air for 24 hours prior to test initiation. The airflow was controlled by flowmeters and regulated to 40 mg/l for each bottle. Check valves were inserted in the lines between the gas washing bottles and the test bottles.

After the bottles were purged with CO2-free air, 500 ml of mineral medium was added to two bottles that served as inoculum blanks (no test material). Sodium benzoate (103 ml of stock solution, for a concentration of 20.0 mg carbon/l) and 397 ml of mineral medium was added to another bottle. Two bottles containing test material were prepared (one containing 0.0836 g of test material and 500 ml of mineral medium, for a concentration of 20.1 mg carbon/l and another containing 0.0846 g of test material and 500 ml of

mineral medium, for a concentration of 20.3 mg carbon/l). The final bottle received 0.0836 g test material, 103 ml of the sodium benzoate stock solution and 397 ml of the mineral medium (20.1 mg carbon/l test material and 20.0 mg carbon/l reference material, for a total of 40.1 mg carbon/l). Each bottle was capped with a silicone stopper. Inlet and outlet tubing was built into each stopper. All of the bottles were placed in an environmental chamber maintained at 22 +/- 2 degrees C, and aerated through the inlet tubing. The medium in each bottle was continuously stirred with a magnetic stirrer.

The evolved CO2 gas that came out of each bottle was trapped in a series of three gas-washing bottles containing 100 ml of 0.0125 M Ba(OH)2. The amount of unreacted Ba(OH)2 was quantified by titrating with a HCl solution to the phenolphthalein endpoint. During the first week of the study, CO2 was analyzed every 2-3 days. After the first week, the sampling schedule was changed to once every 3-5 days. The test was terminated on day 28. On day 28, aeration was stopped, the lids on the bottles were opened and 1 ml of concentrated HCl was added to each bottle to release any CO2 remaining in the test vessel. Final titrations were made on day 29.

The percent biodegradability at each time interval was calculated for each test condition using the following equation: percent biodegradability = cumulative CO2 produced (mg)/ThCO2 (mg) x 100, where ThCO2 (mg) = carbon added (mg) x 44 mg as CO2/12 mg as carbon. The amount of CO2 produced at each time interval = K(50-Vt), where K = 1.10 (the constant which converts volume of HCl titrated to mg CO2 produced), 50 = volume (ml) needed to titrate 100 ml Ba(OH02 and Vt = volume (ml) HCl titrated into the test solution. The amount of CO2 produced in the inoculum only control was subtracted from that produced by the test or reference materials to obtain the amount of CO2 produced from these materials.

Test substance : The test material was Cyanox 1790, used as supplied by the manufacturer. According to the MSDS, the purity of this material is 93.7-98.9%. Impurities are not listed on the MSDS.

Reliability : (2) valid with restrictions
The concentration of material tested was toxic to the inoculum. The actual concentration used was not confirmed analytically, and the material is insoluble in water at the concentrations tested.

Flag : Critical study for SIDS endpoint

14.04.2005 (1)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : other
Exposure period : at °C
Concentration :
Elimination :
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : The CAS No. 40601-76-1 was inputted into the model. The estimate is based on the presence of three phenol groups on the molecule.

Result : The log of bioconcentration factor [calculated by EPIWIN BCF (v2.14)] is log BCF = 0.500. The BCF is 3.162.

Reliability : (2) valid with restrictions
Data were obtained by EPIWIN modeling.

14.04.2005 (6)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 100
LC50	:	> 100
Limit test	:	yes
Analytical monitoring	:	no
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	None of the control or treated fish died or exhibited signs of toxicity. The No effect concentration (NOEC) and 96-hour LC50 values are therefore > 100 mg whole material/l. The temperature ranged from 14.2 - 14.6 degrees C during the test. The dissolved oxygen concentrations in the test water ranged from 10.4 to 10.6 mg/l (102-104 % of saturation) at the beginning of the test and 8.4 to 8.8 mg/l (82-86% of saturation) for the remainder of the test. pH values ranged from 7.4 to 7.6 at the beginning of the test and were all 7.0 at the end of the test.
Test condition	:	Juvenile rainbow trout (4.0 +/- 0.23 cm avg length and 0.8569 +/- 0.12 g average wet weight) were acclimated for 12 days before treatment. They were maintained in filtered laboratory freshwater at a temperature of 13.0 to 15.0 degrees C. They were fed a diet of salmon starter up to 24 hours before treatment. Mortality during the 7-day period prior to testing was 0%. The dilution water was a moderately hard freshwater, which originated from Jupiter Florida. It was treated by vigorous aeration, filtered to 20 microns, passed through activated carbon and re-aerated prior to use. The hardness (as calcium carbonate), alkalinity (as calcium carbonate) and specific conductivity were 90 mg/l, 31 mg/l and 377 microSiemens, respectively. Chemical characteristics were listed in an Appendix to the study. A preliminary 96-hr range-finding test was conducted at nominal concentrations of 0.0 (control), 0.01, 0.10, 1.05, 10.0 and 100 mg whole material/l. Five fish were tested for each condition. None of the fish died in the experiment, and the material was insoluble at each concentration used. Due to the insolubility of the test material in the preliminary test, the definitive test was initiated as a limit test with 100 mg whole material/l. Each of three replicates was spiked by direct addition of the test material (0.9 g) into 9 liters of dilution water. The replicate test chambers were placed onto mechanical stir plates with rotating stir bars. The test material was spiked directly into the vortex and allowed to mix for approximately 25 minutes. The liquid in each of the test chambers was then filtered through a Gelman minicapsule (0.45 microns) Versapore membrane filter. The test filtrate (which appeared clear) was transferred into glass test chambers, placed onto a 15 degrees C water table, and allowed to reach the desired test temperature (15 +/- 1 degrees C). Concurrent controls also were prepared in triplicate. After initial water quality parameters (temperature, pH, dissolved oxygen) were measured, 7 fish (one or two at a time) were added to each test

chamber (loading was 0.67 g fish/l). The test chambers were 10 liter glass tanks (43 cm x 23 cm x 14 cm) containing 9 liters of dilution water and providing a final water depth of 10 cm. All chambers were covered to reduce evaporation. The chambers were placed in a water bath and maintained under a photoperiod of 16 hours light/8 hours darkness (at 6-7 micromoles/m2/sec light intensity). Fish were not fed and water was not aerated during the test. Survival of fish and water quality were monitored daily. The test was terminated after 96-hr evaluations were performed.

Test substance : The test material was Cyanox 1790, used as supplied by the manufacturer. According to the MSDS, the purity of this material is 93.7-98.9%. Impurities are not listed on the MSDS.

Reliability : (2) valid with restrictions
The actual concentration of dissolved test material was not measured analytically.

Flag : Critical study for SIDS endpoint

14.04.2005 (13)

Type : other
Species :
Exposure period : 96 hour(s)
Unit : mg/l
Limit test :
Analytical monitoring : no
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Inputs to the program are the CAS No. 40601-76-1, a measured water solubility of 0.02 mg/l and a measured melting point of 160.5 degrees C.

The EPIWIN ECOSAR program uses imides and phenols as model analogs for this substance.

Result : The LC50 values are 3.08 E-8 mg/l based on the imides class and 6.79 E-7 mg/l based on the phenol class.

Reliability : (2) valid with restrictions
Data were obtained by EPIWIN modeling.

14.04.2005 (7)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = 1000
EC50 : > 1000
Limit Test : yes
Analytical monitoring : no
Method : OECD Guide-line 202
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : The rate of mortality in the controls was 5 % (1/20) at 24 hours, and 10% (2/20) at 48 hours. For treated organisms, the mortality rate was 10% at both 24 and 48 hours (2/20). The no effect concentration (NOEC) and LC50 value at 48 hours were 1000 mg whole material/l and > 1000 mg whole material/l, respectively.

The temperature ranged from 20.1 - 20.9 degrees C during the test. The dissolved oxygen concentrations in the test water ranged from 8.2 - 8.8 mg/l (92-98 % of saturation) at the beginning of the test and 8.0 - 8.4 mg/l (89-93% of saturation) for the remainder of the test. pH values ranged from 7.1 - 7.3 at the beginning of the test and from 7.2 - 7.3 at the end of the test.

Test condition : Daphnia magna used in the test were progeny from animals obtained from Aquatic Research Organisms, Hampton New Hampshire in August 2000. A subculture of adults was isolated from these cultures and maintained on site prior to testing. The cultures were fed the green algae, Selenastrum capricornutum and a solution prepared from yeast, cereal leaves (Cerophyll) and trout chow daily. Less than 24 hours prior to testing, the adults were placed in food-free dilution water. Neonates (< 24 hours old) were collected on July 2, 2002. These organisms were cultured and isolated in moderately hard fresh water (pH 6.5 - 8.0, temperature 20 +/- 2 degrees C), which originated from Jupiter Florida. Water was treated by vigorous aeration, filtered to 20 microns, passed through activated carbon and re-aerated prior to use. The hardness (as calcium carbonate), alkalinity (as calcium carbonate) and specific conductivity were 76 mg/l, 31 mg/l and 407 microSiemens, respectively. Chemical characteristics were listed in an Appendix to the study. No ephippia were produced during culture and the organisms appeared in good physical condition at test initiation.

A 48-hr preliminary range-finding test was conducted at nominal concentrations of 0.0 (control), 0.2, 1, 11, 99 and 1000 mg whole material/l. Five Daphnia were tested for each condition. None of the organisms exposed to concentrations < 1000 mg/l died in the experiment. Twenty percent of Daphnia exposed to 1000 mg/l died. The test material was insoluble at each concentration used.

Due to the insolubility of the test material in the preliminary test, the definitive test was initiated as a limit test with 1000 mg whole material/l. Test water was prepared by directly adding the test material (0.5 g) to 0.5 liters of dilution water. The test vessel was placed onto a mechanical stir plate with a rotating stir bar. The test material was spiked directly into the vortex and allowed to mix for approximately 30 minutes. The liquid was then filtered through a Gelman minicapsule (0.45 microns) Versapore membrane filter. The test filtrate (which appeared clear) was transferred into a glass test chamber, placed onto a 20 degrees C water table, and allowed to reach the desired test temperature (20 +/- 1 degrees C). A control solution containing dilution water only also was prepared.

After initial water quality parameters (temperature, pH, dissolved oxygen) were measured, 5 organisms (one or two at a time) were added to each test chamber. Four replicate chambers were established for the control and test solutions (for a total of 20 control and test organisms). The test chambers were 50-ml glass vials (2.5 cm x 9.5 cm) containing 50 ml of dilution water or test solution (which provided a final water depth of 9.5 cm). All chambers were covered to reduce evaporation. The chambers were placed in a water bath and maintained under a photoperiod of 16 hours light/8 hours darkness (at 9.5-10.6 micromoles/m2/sec light intensity). Organisms were not fed during the test and the water was not aerated. Survival and condition of Daphnia and water quality were monitored daily. The test was terminated after 48-hr evaluations had been performed.

The no effect concentration (NOEC) and EC50 values were to be calculated using ToxCalc Comprehensive Toxicity Data Analysis and Database Software (Version 5.0, 1996).

Test substance : The test material was Cyanox 1790, used as supplied by the manufacturer. According to the MSDS, the purity of this material is 93.7-98.9%.

Impurities are not listed on the MSDS.

Reliability : (2) valid with restrictions
The actual concentration of dissolved test material was not measured analytically.

Flag : Critical study for SIDS endpoint

14.04.2005 (14)

Type : other
Species :
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = .00014 calculated
Analytical monitoring : no
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Inputs to the program are the CAS No. 40601-76-1, a measured water solubility of 0.02 mg/l and a measured melting point of 160.5 degrees C.

EPIWIN ECOSAR program chooses phenols as the model analog for this substance.

Reliability : (2) valid with restrictions
Data were obtained by EPIWIN modeling.

14.04.2005 (7)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species :
Endpoint : other
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 6.14 E-10 calculated
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Inputs to the program are the CAS No. 40601-76-1, a measured water solubility of 0.02 mg/l and a measured melting point of 160.5 degrees C.

EPIWIN ECOSAR Program chooses phenols as the model analog for this substance.

Reliability : (2) valid with restrictions
Data were obtained by EPIWIN modeling.

Flag : Critical study for SIDS endpoint

14.04.2005 (7)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	> 10000 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male
Number of animals	:	10
Vehicle	:	other: 0.25% agar and 0.10% Tween 80
Doses	:	10000 mg/kg
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	None of the animals died and there were no signs of intoxication. Animals gained an average of 106 g over the course of the study. All organs appeared normal at necropsy.
Test condition	:	Ten nonfasted male Harlan-Wistar albino rats (90-120g) were treated with a single oral dose of 10.0 g/kg. The dosing solution contained 0.2 g test material in a total volume of 1 ml of 0.25% agar and 0.10% Tween 80. Animals were observed for toxicity over a period of 14 days, after which they were euthanized and subjected to gross necropsy.
Test substance	:	The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the purity of the material was 95% and the melting point was 145-148 degrees C. Impurities were not listed.
Reliability	:	(2) valid with restrictions Females were not tested.
Flag	:	Critical study for SIDS endpoint

14.04.2005 (2)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	> 20 mg/l
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	4 hour(s)
Method	:	other
Year	:	2001
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	Value was estimated by an unknown method.
Reliability	:	(4) not assignable No test conditions were noted.

14.04.2005 (3)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 5000 mg/kg bw
Species	:	rabbit
Strain	:	other: albino
Sex	:	male
Number of animals	:	5
Vehicle	:	water
Doses	:	5000 mg/kg
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	There were no deaths or signs of intoxication or irritation. Animals gained an average of 264 g over the course of the study. There were no remarkable necropsy findings.
Test condition	:	Test material was applied to clipped skin of 5 male albino rabbits (avg. wt 2492 g). The material that was applied was weighed onto Vinylite and moistened with a volume of water equal to two times the weight of the test material). The test site was then covered (the material used was not listed). Animals were observed for 14 days, after which they were weighed, euthanized and subjected to gross necropsy.
Test substance	:	The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the purity of the material was 95% and the melting point was 145-148 degrees C. Impurities were not listed.
Reliability	:	(2) valid with restrictions Females were not tested.

14.04.2005 (2)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	human
Concentration	:	2.5 %
Exposure	:	Semiocclusive
Exposure time	:	
Number of animals	:	100
Vehicle	:	petrolatum
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: modified Draize-Shelanski Repeat Insult Patch Test
Year	:	1976
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	It is peculiar that the results state that there were 7 days between induction and challenge, and results of 0 are also listed for days 8, 9, and 10 after induction.
Result	:	All subjects had scores of 0 1-10 days after induction and 24 and 48 hours after challenge. The material was therefore neither irritating nor sensitizing.
Test condition	:	One hundred healthy adults (61 females, 39 males, 18-50 years of age, 58

black, 2 Puerto Rican, 40 white) served as subjects for the study. A 15 mm patch of test material (2.5% in white petrolatum) was applied to patch sites on the backs or volar forearms of the subjects for 10 alternate-day 24 hour periods under occlusion. Following a seven day test period (in which subjects were evaluated daily), 15 mm challenge patches (1.0% in white petrolatum) were applied in the same manner to fresh sites on the backs or volar forearms of all subjects for 24 hours. Challenge sites were read on removal of the patch and 24 hours thereafter.

Test substance : The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the melting point was 159-161 degrees C. Impurities were not listed.

Reliability : (2) valid with restrictions
The scoring system was not described.

14.04.2005 (16)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	
Dose	:	100 other: mg
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	
Result	:	not irritating
Classification	:	
Method	:	other: modified FHSA procedure
Year	:	1973
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Remark : It is unclear how the eyes were scored. No scores were provided.

Result : There was no irritation of the cornea, iris or conjunctiva at 24, 48 or 72 hours in any of the eyes.

Test condition : Test material (100 mg) was applied to one eye of each of 6 rabbits. Eyes were evaluated 24, 48 and 72 hours after treatment.

Test substance : The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the purity of the material was 95% and the melting point was 145-148 degrees C. Impurities were not listed.

Reliability : (2) valid with restrictions
The study is not well documented.

14.04.2005 (2)

5.3 SENSITIZATION

Type	:	other: modified Draize-Shelanski Repeat Insult Patch Test
Species	:	human
Concentration	:	1st: Induction 2.5 % 2nd: Challenge 1 % 3rd:
Number of animals	:	100
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other
Year	:	1976
GLP	:	no data

Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	It is peculiar that the results state that there were 7 days between induction and challenge, and results of 0 are also listed for days 8, 9, and 10 after induction.
Result	:	All subjects had scores of 0, 1-10 days after induction and 24 and 48 hours after challenge. The material was therefore neither irritating nor sensitizing.
Test condition	:	One hundred healthy adults (61 females, 39 males, 18-50 years of age, 58 black, 2 Puerto Rican, 40 white) served as subjects for the study. A 15 mm patch of test material (2.5% in white petrolatum) was applied to patch sites on the backs or volar forearms of the subjects for 10 alternate-day 24 hour periods under occlusion. Following a seven day test period (in which subjects were evaluated daily), 15 mm challenge patches (1.0% in white petrolatum) were applied in the same manner to fresh sites on the backs or volar forearms of all subjects for 24 hours. Challenge sites were read on removal of the patch and 24 hours thereafter.
Test substance	:	The test material was Antioxidant A-1790 (Cyanox 1790), used as supplied by the manufacturer. According a data sheet supplied with the study, the melting point was 159-161 degrees C. Impurities were not listed.
Reliability	:	(2) valid with restrictions The scoring system was not described.

14.04.2005 (16)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	30 days
Frequency of treatm.	:	continuous
Post exposure period	:	not applicable
Doses	:	0.5, 1.0 and 2.0 % (600, 1200 and 2380 mg/kg/day in males and 610, 1190 and 2480 mg/kg/day in females)
Control group	:	yes, concurrent no treatment
NOAEL	:	= 1 %
LOAEL	:	= 2 %
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	This was a range-finding study for a 90-day study. Therefore, it was not designed to be an in-depth examination. It is likely that the increased relative liver weight in the high dose females is due to reduced body weight. The authors did not consider the decreased body weight of high dose females or the changes observed in the liver of one high dose male to be indicative of a treatment-related effect. They therefore determined that the no effect level was 2%. However, it is interesting to note that high dose females had lower body weights but increased food consumption. Therefore, it appears that the material did have an effect on body weight at the highest dose. Based on this finding, the NOAEL assigned by the summary preparer was 1%. It was noted in the text that diarrhea occurred in high dose (as well as low dose animals). No explanation was given for this observation, and it was not considered to be significant by study personnel. Based on the results, doses of 100, 400 and 1600 mg/kg were assigned for

the 90-day study. However, the highest dose actually used in the 90-day study was 400 mg/kg.

Result : None of the animals died. Rats receiving test material exhibited diarrhea (low and high-dose animals) and irritation of the face, ears and/or stomach (mid and high dose animals). Because similar irritation is occasionally observed in Sprague-Dawley rats it was not attributed to administration of test material. Alopecia was noted in controls as well as treated animals. A significant effect of treatment on food consumption at week 4 was noted in females. There was a trend towards increased food consumption in all treated groups, with the value for the high-dose females significantly different from control. The average values for food consumption of females treated with 0, 0.5, 1.0 and 2.0% test material were 118, 133, 131 and 138 g/week respectively. The authors concluded that the increase in food consumption was not related to administration of test material.

Although the authors stated there was no effect on body weight, there was a trend towards decreased terminal body weight in females at the two highest doses, with the average value for the high dose group significantly different from control. The average values for terminal body weight of females treated with 0, 0.5, 1.0 and 2.0% test material were 199, 208, 173 and 159 g/week respectively.

There was no effect of treatment on absolute organ weights. However, in females, average relative liver weights of animals treated with 0.5% (4.98), 1.0% (5.97) and 2.0% (6.98) and average relative kidney weights of animals treated with 2.0% test material (1.207) were significantly different from control values for liver (4.67) and kidney (0.831).

The only significant gross observation seen at necropsy was discoloration of the liver of 2 high dose males and 2 high dose females. No histopathological changes were observed in 3/4 of these rats. One high dose male exhibited minimal intralobular scattered foci of extra medullary hematopoesis and mononuclear infiltrates. Infrequent degenerating individual hepatocytes were associated with the mononuclear infiltrates. It was concluded that the effects on the liver were not due to test material.

Test condition : Forty healthy CD rats (<= 4 weeks past weaning and < = 100 g) were acclimated for 7 days prior to treatment. The rats were housed individually and provided food and water ad libitum. Rats were randomly allocated to 4 groups of 5/sex. The groups were given diet containing 0 % (control), 0.5, 1.0 or 2.0 % test material for 30 consecutive days. Details about how the diets were prepared were not present.

All animals were observed daily to detect possible signs of toxicity. Food intake and body weight were measured weekly. Necropsies were performed on all animals at study termination. At necropsy, all animals received a thorough examination including all body surfaces, both internal and external, subcutaneous tissues, and the following organs: adrenal, aorta, urinary bladder, bone, bone marrow, cerebellum, cerebrum, colon, esophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, skeletal muscle, sciatic nerve, ovary, pancreas, parathyroid, pituitary, prostate, salivary gland, seminal vesicle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea and uterus. The liver and kidneys (combined) were weighed. The animal carcass also was examined for lesions and other irregularities. The aforementioned organs were preserved in buffered 10% formalin and the livers of 2 high dose male and female rats were examined histologically.

Data were analyzed using a parametric analysis of variance (randomized block design) and Student's t tests. The level of significance was $p < 0.05$.

Test substance : The test material was Cyanox 1790. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the

purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

Reliability : (2) valid with restrictions
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. Clinical chemistry, urinalysis and hematological parameters were not measured.

Flag : Critical study for SIDS endpoint

14.04.2005 (19)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	continuous
Post exposure period	:	not applicable
Doses	:	25, 100 and 400 mg/kg
Control group	:	yes, concurrent no treatment
NOAEL	:	= 400 mg/kg bw
Method	:	other
Year	:	1977
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Result : One animal in the mid-dose group (100 mg/kg) died after 12 days of treatment. Study personnel did not attribute this death to administration of test material. Animals were generally healthy during the study, but exhibited alopecia around the nares and paws, diarrhea, watery eyes (animals that were bled, only) and encrustment around the nares. Study personnel attributed these signs to ingestion of a powdered food and did not consider them to be related to test material. Other than week one (where females treated with 25 mg/kg test material ate less food than females in other groups) and week 3 (when males in the 25 mg/kg group ate less food than males in the other groups), there was no difference between food intake of treated and control animals. There was no effect of treatment on weight or weight gain.

Gamma-glutamyl transpeptidase (GGTP) values in males treated with 400 mg/kg were higher than control at 90 days (4.1 vs. 2.6 IU/l), but were within historical limits. The elevation was due to 2/5 males that had GGTP values that were twice the values of the other males. Glucose values in females treated with 100 and 400 mg/kg (163 and 162 mg/dl, respectively) were lower than controls (209 mg/dl) at 90 days but were within the range of historical values (90-284 mg/dl). The significant differences in glucose between high and mid dose females and controls were considered to be a result of some unusually high values in the controls and were not considered to be related to test material.

At 90 days, there was a trend toward increased red blood cells in low (8.27 x 10E6/mm3) and mid-dose animals [(8.63 x 10E6/mm3, value significantly different from study control (7.99 x 10E6/mm3) and historical controls]; however, counts in high dose animals (8.04 x 10E6/mm3) were similar to controls. Since the increase was not dose-related, this observation was thought to be artifactual in nature and not related to administration of test material.

There was no effect of treatment on urinalysis, organ weights, or gross pathology. One of the males with a high GGTP value had focal perivascular and periductal mononuclear leukocyte infiltration in the liver, but the other animal did not demonstrate any liver pathology. Livers of other males in the high dose group had histopathology similar to that of

controls. Other mild inflammatory lesions characterized by leukocytic infiltration also occurred in the lungs, liver and kidneys of a few animals in each group. All changes were considered to be spontaneous and not related to administration of test material.

Test condition : COBS Sprague-Dawley rats (80 per sex) were acclimated for 8 days before treatment. They were then assigned to 4 test groups [0 mg/kg bw (control diet), or 25, 100 or 400 mg/kg bw test material] of 20 per sex per group by weight stratification. At time of assignment, all animals were 4 weeks old. Weights of male and female animals ranged from 69-100 g and 64-95 g, respectively. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-75 degrees F and 40-50% relative humidity, with 10 exchanges of air/hour. The light cycle was 12 hr light/12 hours dark.

Test diets were prepared by mixing the appropriate amount of test material with equal amounts of ground Purina Lab Chow in a high speed blender (blending time was not noted). After blending, an additional 2 parts of ground lab chow was mixed by hand into the blended material. After this step, an additional amount of ground lab chow was added to give the appropriate dose level (25 mg/kg bw, 100 mg/kg bw or 400 mg/kg bw). The diet was then placed in a twin-shell mixer and mixed until the test material was distributed throughout the diet. The formula used to determine the amount of material added to feed was as follows: mean body weight x dietary level x 7 days/mean weekly food consumption = mg test material/kg feed. Fresh diets were prepared at least once per week. Doses were adjusted weekly on the basis of the weight and food consumption for the preceding week. Doses were not adjusted for purity of the material.

Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. Animals selected for clinical chemistry, hematology and urinalysis (5 per sex per group) were determined by protocol and a table of randomization. Clinical chemistry (gamma-glutamyl transpeptidase, glucose, glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase, and blood urea nitrogen), hematology (erythrocyte count, hematocrit, hemoglobin, differential and total leukocyte count, and platelet count) and urinalyses (appearance, glucose, microscopic examination of sediment, pH, protein and specific gravity) were performed on the same animals at appropriate time intervals (day 90 for clinical chemistries, days 45 and 90 for hematologies). Animals were fasted overnight before blood was collected from the orbital sinus on day 45 and from an unknown site on day 90. Urine also was collected on days 45 and 90. Organ weights (brain, gonads, heart, kidneys and liver) were measured in all animals that survived to day 90.

At study termination, all animals were weighed. They were then euthanized. The urogenital orifaces, tail, each pinna, eyes and external auditory meatus were examined visually and by palpatation for distortions. All subcutaneous tissues were examined including regional lymph nodes, mammary and salivary glands. Abdominal contents, and the brain, pituitary gland and cranial nerves also were examined grossly. The following tissues were taken and preserved for possible histologic evaluation: adrenal, aorta, urinary bladder, bone, bone marrow, cerebellum, cerebrum, pancreas, pituitary, skin , stomach, thyroid, colon, esophagus, eye, heart, ileum, jejunum, kidney, prostate, salivary gland, spleen, testes, tongue, uterus, liver, lung, lymph node, mammary gland, muscle (skeletal), sciatic nerve, ovary, parathyroid, seminal vesicle, spinal cord, thymus and trachea. Any organ or tissue exhibiting a lesion was noted and the lesion was taken for histopathological examination. Complete histopathology was performed on 10 animals/sex/group from the control and high dose groups. These animals were selected randomly from a table. Microscopic examination of the heart, liver, lungs and kidneys was performed on all

additional animals that survived to termination.

Data for food intake, weight gain, clinical chemistries, hematologies, urinalyses, and organ weights were analyzed using a blocked 2-way analysis of variance that compared data between and within groups. If F values were significant, data were compared using Dunnett's t-test. The level of significance is $p < 0.05$.

Test substance : The purity of the test material was 90.0%. Impurities were not listed.

Conclusion : The changes that were observed (elevated gamma-glutamyl transpeptidase in high dose males, decreased glucose in females, and increased red blood cell counts in mid-dose males at 90 days) were not considered to be related to test material since they were not dose-dependent and were within normal limits. Therefore, authors concluded that the "no effect" dose was 400 mg/kg bw.

Reliability : (2) valid with restrictions
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. More hematological, clinical chemistry and urinalysis parameters are measured in guideline studies. A high enough dose to produce a significant toxicological effect was not used.

14.04.2005 (21)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 15-28 days (males), 40-53 days (females)
Frequency of treatm. : continuous
Post exposure period :
Doses : 1000, 10000 and 20000 ppm
Control group : yes
NOAEL : = 1000 ppm
LOAEL : = 10000 ppm
Method : other: OECD Guideline 421
Year : 2005
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : This study only contains methods and results pertinent to repeated dose toxicity of the parental animals. Reproductive/developmental effects are listed under Sections 5.8 and 5.9, respectively.

Doses of 1000, 10000 and 20000 ppm are equivalent to 69, 651 and 1294 mg/kg/day in males and 77, 782 and 1558 mg/kg/day in females.

Result : Mortality and Clinical Signs: There were no treatment-related mortalities. One control female was killed in extremis due to an injury to the tail. No other macroscopic abnormalities were observed at the post mortem examination (with the exception of the skin lesions noted below).

At 20000 ppm, three males and all females showed scabs and fur loss on the top of the head and/or around the snout from Day 22 of treatment onwards. These findings were noted in all females, but in no males treated with 10000 ppm. For all but one 10000 ppm female, these signs persisted until completion of the study. Scabbing also was noted on the abdomens of two females treated with 20000 ppm. The scabbing was considered to be an adverse effect of treatment due to its severity. There were no clinical signs of toxicity or irritancy in animals treated with 1000 ppm. One control female had approximately 2 inches of skin missing from the end of the tail, exposing underlying tissue.

Body weight and food consumption: There was no significant effect of treatment on body weight or body weight gain during maturation, gestation

or lactation. There was a statistically significant ($p<0.05$) increase in the amount of food eaten during the first week of treatment in male rats treated with 20000 and 10000 ppm compared to controls (31 g/rat/day in each group vs. 29 g/rat/day in control). This was considered to be incidental due to the lack of a dose-related response and comparable changes in female food consumption. Daily visual inspection of water bottles revealed no intergroup differences in water consumption.

Organ weight, gross and histopathology data: There was no effect of treatment on parental organ weight. At 20000 ppm, fur loss and scabbing was observed in all females and 3 males (predominantly on the head). Similar skin lesions were noted on the abdomens of two females at this dose level. Fur loss and scabbing on the head also were observed in 9 females treated with 10000 ppm. At 1000 ppm, one male showed sloughing of the glandular region of the gastric epithelium. This was not considered to be related to treatment due to the lack of similar findings at higher doses. One control female had approximately two inches of skin missing from the tip of the tail exposing underlying tissue.

Acanthosis, occasionally with focal epithelial ulceration and overlying scab formation, was observed in 9/10 females dosed with 20000 ppm ($p<0.001$) and 8/9 females dosed with 10000 ppm ($p<0.01$). One male at 20000 ppm also was affected. All remaining morphological changes were similar in incidence and degree of severity to controls and were commonly observed in laboratory maintained rats of the same age and strain. Specifically, no significant histopathological abnormalities were observed in the gonads or other accessory sex organs of control or treated rats.

Test condition : Date of study: June 18, 2004 - February 11, 2005

Animals: Male and female Sprague-Dawley Crl:CD® (SD)IGS BR rats were obtained from Charles River (UK) Limited, Manston Road, Margate, Kent. They were examined on the day of receipt and acclimated for 7 days. A total of 40 animals/sex were accepted into the study. The males and females weighed 340-394 g and 209-243 g at the start of the study, respectively.

Animals were housed in groups of five by sex in polypropylene cages with stainless steel grid floors and tops, suspended over paper-line polypropylene trays (except during mating and gestation). One male and one female were housed in similar cages during mating. After evidence of successful mating, males were returned to their original cages. The females were then housed individually in cages with solid floors, soft wood chip bedding and stainless steel tops.

Animals were housed in an environmentally-controlled room, under a 12 hour light/dark cycle. The temperature and relative humidity were maintained to operate within a target range of 21 +/- 2 degrees C and 55 +/- 15 %. Air was changed at least 15 times per hour. Certified feed and water were supplied ad libitum. No contaminants were present in food and water at levels sufficient to affect the outcome of the study.

Test material: The test material was incorporated into the diet at concentrations of 1000, 10000 and 20000 ppm by mixing a known amount of material with a known amount of basal laboratory diet for 20 minutes at a constant speed in a Hobart mixer. Samples were analyzed for stability and homogeneity by high performance liquid chromatography (with an external standard) before the beginning of the study. Samples for homogeneity analyses were taken from the middle and two opposite sides of the container, in triplicate. Samples for stability analyses were sampled and analyzed initially and after storage at ambient temperature in the dark for 14 days. Results of the analyses showed that the material was homogeneous in the diet and stable for at least 14 days. Dietary

admixtures were prepared prior to treatment and weekly thereafter. Samples of each admixture were taken every 14 days and analyzed for concentration of test material. All admixutures were within acceptable limits of the desired concentration.

Study design: The animals were randomly allocated by weight to 4 groups of 10 animals per sex (F0 animals) receiving either 0 (vehicle), 1000, 10000 or 20000 mg/kg/day test material. Animals in each group were uniquely identified. Test material administration to F0 males and females began 14 days prior to mating and continued through mating. Females continued receiving test material to postnatal day 5.

Following 14 days of dosing, 10 F0 males were randomly paired on a 1:1 basis with 10 F0 females from the same group for up to 14 days. Following positive identification of mating (presence of a copulatory plug in the vagina or on the tray beneath the cage or the presence of sperm in a vaginal smear following vaginal lavage), the males were returned to their cages. Following mating, all F0 females were allowed to deliver naturally and rear their young to postnatal day 5 (the scheduled day of necropsy).

All F0 animals were observed twice daily for mortality and morbundity (once daily on weekends). Clinical observations were recorded daily. Male body weights and food consumption per cage were recorded weekly throughout the study. Body weights and food consumption per cage of females were recorded weekly until mating. After mating, body weights of females were recorded on gestation days 0, 7, 14, and 20 and on lactation days 1 and 4, and food consumption of females was determined for gestation days 1-7, 7-14 and 14-20. Female food consumption also was recorded for the period covering lactation days 1-4.

Males were euthanized after confirmation of successful mating. All surviving adults (including non-fertile animals) and offspring were euthanized on day 5 postpartum. All animals were examined microscopically for internal and external abnormalities. The testes and epididymides of all adult males were weighed and preserved in Bouins solution. The coagulating glands, epididymides, prostate, seminal vesicles, testes, pituitary, ovaries, uterus/cervix, vagina and skin (from the top of the head) from the high dose and control adult males and females were fixed, processed and examined microscopically by a pathologist.

Statistical evaluations: Data were processed to give group mean values and standard deviations. Adult body weight, body weight gain and food consumption and organ weight data were analyzed for homogeneity using Bartlett's' test, followed by a one-way analysis of variance (ANOVA). Data that were not homogeneous were subsequently analyzed using a t-test (assuming unequal variances). Dunnett's multiple comparison method was used to analyze data that were homogenous. Relative organ weights were analyzed using the Kruskal-Wallis non parametric rank sum test. Histopathological lesions that occurred at an overall frequency of 1 or greater were analyzed using a chi-squared test. Severity grades were analyzed using a Kruskal-Wallis one-way non-parametric ANOVA. Significant differences were reported at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ level (if present).

Test substance : The test material contained 96-97% 1,3,5-Tris[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-xylyl]methyl]1,3,5-triazine-2,4,6(1H,3H,5H)-trione (CAS No. 40601-76-1). Impurities were not listed.

Reliability : (2) valid with restrictions
The study is valid without restriction for the reproductive/developmental endpoints. However, some of the parameters measured in guideline repeated dose studies (hematologies, clinical chemistries and complete organ histopathology) were not performed. Therefore, a rating of (2) is appropriate for the repeated dose toxicity endpoint.

24.05.2005 (17)

Type	:	Sub-chronic
Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	oral feed
Exposure period	:	13 weeks (91 days)
Frequency of treatm.	:	1 hour/day
Post exposure period	:	not applicable
Doses	:	46 mg/kg/day
Control group	:	yes, concurrent no treatment
NOAEL	:	= 46 mg/kg bw
Method	:	other
Year	:	1983
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Result : None of the controls or animals treated with CL 226,034 died. Clinical signs such as unilateral or bilateral serous ocular discharge, undigested food (occasionally mixed with mucoid substance) found in the tray after feeding, loose feces (occasionally mixed with mucoid substance) found in tray before and after feeding, redness of the pinnae, and areas of skin redness and partial alopecia were noted in both control and treated animals at similar incidences.

Throughout the treatment period, the mean body weights of CL 226,034-treated dogs of both sexes were consistently lower than the controls, but none of the values were statistically significant. The mean body weight gains of control males and females were 2.4 and 2.3 kg, respectively, and treated males and females gained an average of 2.0 and 1.7 kg, respectively. Throughout the experiment, the average food intake of treated animals was slightly but consistently lower than that of treated dogs. None of the values were significantly different in males. Average food intake of treated females was significantly different from controls at week -3 (217.2 g/dog/day in control vs. 160.4 g/dog/day in treated), week 1 (310.7 g/dog/day in control vs. 275.5 g/dog/day in treated), and week 2 (330.6 g/dog/day in control vs. 288.3 g/dog/day in treated).

The test material was stable in the diet for 14 days and was distributed homogeneously. The amount of test material intake in males and females ranged from 88.4-103.7% and 99.4 - 110.0% of target, respectively. The mean amount of CL 226,034 intake for the entire feeding period was 97.5 +/- 4.41% of the desired amount for males and 103.8 +/-3.64% for females.

The ophthalmologic examinations of 2 female dogs (one control and one treated) were abnormal during the test. One control had a right eye with a small focus of tapetal hypereflectivity and pigment clumping lateral to the main retinal vessels at pretreatment, which was not evident at week 6. At week 12, the right eye had a small area of tapetal hypereflectivity that appeared to be a focal inactive scar. In a treated animal that had normal exams at pretreatment and week 6, the right eye had a small hemorrhage in the nerve fiber layer, superolateral to the optic nerve head at week 12. The significance of these findings was not listed.

Throughout the study, there was no consistent effect of test material on any hematological, clinical biochemical or urinalysis parameter. Occasional differences in parameters were judged to be inconsequential since the values were within normally accepted ranges, did not change consistently over time and were not observed in both sexes.

There was no effect of treatment on weight of any organ examined. Gross

pathological findings revealed that 4 dogs (1 male and female in each of the groups) had roundworms in the intestines. Other gross findings were judged to be incidental. Two controls showed excoriation and/or reddening of the skin. Upon histological examination, one of the animals had demodectic mange, and the other showed slight inflammatory changes. Another control dog that did not exhibit gross skin changes also had demodectic mange upon histologic examination.

Histopathological examinations did not reveal any treatment-related effects. One treated male had a small granuloma and a focus of eosinophils in the mesenteric lymph nodes. It was concluded that this was probably associated with parasitic larval migration. Parafollicular cell hyperplasia in the thyroid was found in 3 treated animals and 3 controls. One control and one treated male had macrophages containing a dark brown pigment in the paracortical zone of 1 manidbular lymph node. A focus of capsular thickening seen in the spleen of a treated female was judged to be incidental.

Test condition : Purebred beagle dogs (31/sex, approximately 5 months old) were acclimated for 55 days before use. The supplier had immunized the dogs against canine distemper, infectious canine hepatitis, canine leptospirosis, parovirus and rabies. A veterinarian concluded that they were healthy prior to shipment. The body weight range of the animals just prior to treatment was 5.6 to 9.3 kg for males and 4.5 to 8.5 kg for females. The dogs were housed individually in a room maintained under a 12 hr light/12 hr dark cycle and 12 air changes/hour.

During the acclimation period, the animals were randomly assigned to 7 treatment groups of 4 animals/sex. Five of these groups were going to be treated with a compound B (which was not identified). One group served as a control group, and the other was to be treated with CL226,034 (Cyanox 1790).

Diets containing the test material were prepared weekly by adding the required weight of the test material to known amounts of powdered certified dog chow and mixing for approximately 30 minutes in a Hobart mixer. Homogeneity and adequacy of mixing of the test diets were evaluated by analyzing samples from the top, middle and bottom of the mixer. Stability of the test diet was analyzed immediately after preparation and storage at ambient temperature for 7 and 14 days. Fresh diets were stored at approximately 4 degrees C in labeled airtight plastic containers for the period of use. The concentration of test material in the diets was checked during weeks 1, 2, 4, 8 and 13, immediately after preparation.

From study day -5.1 to 0.1, the dogs were fed 400 g/day of a standard certified commercial dog food. From study day 0.2 to termination, the males were fed 450 g/day and the females 400 g/day. For one hour per day, test animals were fed diet containing test material (compound B or 46 mg/kg/day CL226,034). Fresh water was available ad libitum. There were no contaminants in the food or water that were expected to affect the outcome of the test. All controls and animals treated with CL226,034 were on test for a minimum of 91 days.

Animals were observed for clinical signs twice daily (or more frequently if necessary). Animals that exhibited overt changes in physical appearance or behavior were examined by a veterinary aide. Abnormal animals were examined by a veterinarian. Each animal was weighed weekly prior to and during the treatment period, and just prior to necropsy. Food consumption was determined daily and calculated and documented weekly. An ophthalmoscopic examination was conducted prior to treatment and during weeks 6 and 12 of treatment on all controls and animals treated with CL226,034. Atropine sulfate (1% solution) was administered as a mydriatic, and the conjunctivae, sclera, cornea, iris and fundus were

examined by a veterinary ophthalmologist.

Laboratory investigations (hematologies, clinical biochemistries and urinalysis) were performed on all controls and animals treated with CL226,034 prior to treatment and during weeks 6 and 12. Blood samples were collected from the jugular veins after animals were fasted overnight. Urine samples were collected over the final 17 hours of a 21-hour period food and water deprivation. The hematological parameters measured were hemoglobin, hematocrit, red blood cell count, white blood cell count (total and differential), platelet count, prothrombin time, and Wintrobe's constants). Clinical biochemistries were blood urea nitrogen, glucose, total protein, albumin (A), globulin (G), A/G ratio, serum sodium, potassium, calcium and chloride, creatinine, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (SAP), lactate dehydrogenase (LDH) and total bilirubin. Urinalyses included volume, specific gravity, pH, protein, color and appearance, hemoglobin, nitrite, glucose, urobilinogen, ketones, microscopy of centrifuged deposit and bile pigments.

A complete gross necropsy was performed on each dog that survived to study termination. The animals were euthanized and exsanguinated, and the following organs were dissected and weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes and thyroid lobes (with parathyroids). Paired organs were weighed separately. The following organs were preserved, sectioned, stained and subjected to histopathological examination: adrenals, aorta (thoracic), bone (sternum with marrow, brain (cerebellum, cerebrum and brain stem), cecum, colon, duodenum, epididymides, esophagus, eyes, jejunum, gallbladder, heart, ileum, kidneys, liver (sample from 2 lobes), lungs, lymph nodes (mandibular and mesenteric), mammary gland (inguinal), optic nerves, ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical), spleen, stomach (cardiac region, fundus and pyloric region), testes, thymus, thyroid lobes (and parathyroids), tongue, trachea, urinary bladder, uterus (horns and body), vagina and any abnormalities. Femoral bone marrow smears were prepared for each animal and were stained with May-Grunwald-Giemsa. These smears were to be evaluated if hematological findings were abnormal.

Mean values and standard devotions of data (with the exception of hematological and blood biochemical data) were statistically analyzed using Student's t-test. The probability of t was calculated at the $p < 0.5$ value, and any significant differences were noted.

Hematological and blood biochemical data were analyzed using Bartlett's test followed by Student's t-test when the variance between groups was homogeneous or by the Kruskal-Wallis test when the variance between groups was heterogeneous.

Test substance : The test material was CL 226, 034, which is Cyanox 1790. It was supplied as a white powder from American Cyanamid. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

Reliability : (2) valid with restrictions
Only one dose of material was tested. An additional dose that was expected to produce toxicity should have been tested. Otherwise, the study was comparable to a guideline study.

24.05.2005 (23)

Type : Sub-chronic
Species : dog

Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	continuous
Post exposure period	:	not applicable
Doses	:	7.5, 15 and 30 mg/kg
Control group	:	yes, concurrent no treatment
NOAEL	:	= 30 mg/kg bw
Method	:	other
Year	:	1977
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Remark : The no effect level assigned by study personnel was 30 mg/kg. They did not consider the reduced body weight gains in females to be related to test material because 1) food consumption was comparable between groups and 2) no abnormal clinical signs were noted.

Result : None of the animals died during the study. During the treatment period, variations in stool consistency (soft stool, diarrhea, etc.) were noted intermittently in treated animals. This observation was attributed to the introduction of a foreign material into the GI tract of the beagle and was not considered to be related to the administration of Cyanox 1790 specifically. There were no significant differences in food consumption between controls and animals treated with test material. All animals gained or maintained body weight. There were no significant differences between weights of male control and treated dogs except at week 0 (before treatment). Female dogs treated with any dose of test material had lower body weights than controls throughout the study (including week 0). Weight gains of females decreased in a dose dependent manner, with the average gains for females treated with 0, 7.5, 15, or 30 mg/kg being 1.6, 1.3, 1.1, and 0.7 (units were not listed, but are assumed to be kg).

At the 30 day interval, dogs treated with 7.5, 15 or 30 mg/kg test material (112, 112 and 119 mg/dl in males and 114, 124 and 119 mg/dl in females, respectively) had significantly lower glucose values than dogs in the control group (140 and 133 mg/dl in males and females, respectively). At 90 days, female dogs treated with 7.5 and 30 mg/kg had lower glutamic-oxaloacetic transaminase values (27 and 26 IU/l) than controls (32 IU/l). At 90 days, the erythrocyte count was significantly lower in male dogs treated with 15 mg/kg than controls (5.47 vs. 6.11 x 10E6/mm3, respectively). As all values were within normal limits, these observations were not considered to be related to treatment. No other differences in any clinical chemistry or hematological parameter and no changes in any urinalysis parameter were noted between groups.

There was no effect of treatment on weight of any organ examined or on gross or histopathology. All changes observed were considered to be related to necropsy procedures or a parasitic infection.

Test condition : Thirty two pure bred Beagle dogs (16 per sex) were acclimated for 16 days before treatment. They were vaccinated against canine distemper, hepatitis and leptospirosis by the supplier. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-76 degrees F and 25 - 50% relative humidity. The light cycle was 12 hr light/12 hours dark. Cages were cleaned semi-monthly.

Dogs were assigned to 4 test groups [0 mg/kg bw (control diet), or 7.5, 15 or 30 mg/kg bw test material] of 4 per sex per group one week prior to treatment by weight stratification. On day 1 of treatment, weights and ages of the animals were 5.1 - 6.7 kg and 4 - 5 months, respectively.

Test diets were prepared by geometrically diluting the appropriate amount

of test material with Purina Laboratory Canine Diet in a grounded twin shell blender. The mixing time was not mentioned. Diets containing the least amount of test material were mixed first. The blender was completely cleaned between mixing each dose level. The formula used to determine the amount of material added to feed was as follows: mean body weight (g) x dose (mg/kg bw)/mean food consumption per day (g) = mg test material/kg diet. The desired doses were 7.5, 15.0 and 30.0 mg/kg bw. Doses were not adjusted for purity of the material. Doses were adjusted weekly based on the group body weights of dogs for that week. Diets were prepared weekly.

Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. Clinical chemistry (gamma-glutamyl transpeptidase, glucose, glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase, and blood urea nitrogen), hematology (erythrocyte count, hematocrit, hemoglobin, differential and total leukocyte count, and platelet count) and urinalyses (appearance, glucose, microscopic examination of sediment, pH, protein and specific gravity) were performed on blood and urine collected from all animals after a 16 hour fast during the week prior to treatment, and weeks 8 and 13 of treatment. The site of blood collection was not mentioned.

At study termination (days 90 and 9a), all animals were weighed. They were then euthanized and exsanguinated. The urogenital orifaces, anus, tail, external auditory meatus and skin were examined visually and by palpation for masses. All subcutaneous tissues were examined including regional lymph nodes, mammary and salivary glands. The brain, pituitary gland, spinal cord, eyes and cranial nerves also were examined grossly. The entire gastro-intestinal tract from the cephalad part of the esophagus to the caudal part of the colon was incised, washed with tap water, and the mucosa was subjected to visual examination. The condition of the thoracic viscera was noted including the thymus and lymph nodes. The lungs were removed and all lobes examined, and the heart was excised to permit examination of each chamber. The brain, gonads, heart, kidneys, pituitary, thyroids and liver of all animals were weighed. The liver and kidneys were sectioned at varying intervals.

Small portions of the following tissues were taken and preserved in 10% neutrally buffered formalin for possible histologic evaluation: adrenal, aorta, gall bladder, urinary bladder, bone, bone marrow, cerebellum, cerebrum, colon, esophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, muscle (skeletal), sciatic nerve, ovary, pancreas, parathyroid, pituitary, prostate, salivary gland, skin, spinal cord, spleen, stomach, testis, thymus, thyroid, tongue, trachea and uterus. Sections were prepared at 5 microns from paraffin blocks and stained with hematoxylin and eosin.

Data for food intake, body weight, clinical chemistries, hematologies, and organ weights were analyzed using a randomized block analysis of variance. The level of significance is $p < 0.05$. No statistical evaluations were performed on survival or urinalysis data and incidences of gross or microscopic lesions.

Test substance : The page describing the purity of the test material was missing from the report. However, the purity of the test material used in a rat study that was performed at the same facility around the same time was 90.0%. Impurities were not listed.

Reliability : (2) valid with restrictions
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. More hematological, clinical chemistry and urinalysis parameters are measured in guideline studies. A dose that produced a significant toxicological effect also should have been tested.

24.05.2005 (20)

Type	:	Sub-chronic
Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	oral feed
Exposure period	:	30 days
Frequency of treatm.	:	continuous
Post exposure period	:	not applicable
Doses	:	25, 100, 400 and 1600 mg/kg
Control group	:	no
NOAEL	:	< 400 mg/kg bw
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Remark : On days 22-30, dogs in the 25, 100 and 400 mg/kg/d groups were inadvertently given 2.1 g test material/kg feed (84.0 mg/kg bw), 8.17 g test material/kg feed (333.3 mg/kg bw), and 26.7 g test material/kg feed (1335 mg/kg bw). The LOAEL listed above was assigned by study personnel. However, since all doses appeared to cause histological changes in the liver, the LOAEL appears to be lower than the lowest doses tested (25-84 mg/kg).

Result : None of the animals died. No abnormalities were noted in the general appearance or behavior. Soft feces were noted on various days from days 11-22 in 1/2 animals dosed with all concentrations of test material except the high dose, and 2/2 animals treated with the high dose. The number of days within this interval that the dogs exhibited soft feces increased with increasing concentration.

Both dogs treated with the highest two concentrations of test material (400-1135 and 1600 mg/kg) and one dog in the 100-333.3 mg/kg group lost weight during the study. The other dogs gained weight at an acceptable rate. Weight loss was attributed to be a significant effect of treatment. Food consumption in the highest two dose groups was reduced.

One high dose dog had increased liver, kidney and adrenal weights compared to the other animals and historical controls. Another high dose dog had a slightly increased adrenal weight. One dog in the 100-333.3 mg/kg group had increased kidney weight.

A consistent discoloration and purple stippling of the liver was noted (particularly in the high dose animals). The gall bladder of one dog in the 100-333.3 mg/kg group and another in the 1600 mg/kg group appeared thickened and contained bright yellow bile. The dog in the 1600 mg/kg group that had changes in the gallbladder also had an enlarged spleen and dark colored peribronchial nodes. The kidneys of dogs in all groups except the 25-84 mg/kg group had pale cortexes and dark pink outer medullae. The cardiac muscle of one high dose dog had a greyish tinged appearance.

The liver, kidney, gallbladder and heart of the animals that exhibited gross changes were examined histologically. The heart, gallbaldder and kidneys were normal (with the exception of moderate vacuolization of the proximal tubule epithelium in one 400-1135 ppm female). At 100-333.3 mg/kg and above, there was marked central lobular hepatocyte degeneration. The change was characterized by central lobular fatty degeneration, hydropic swelling of hepatocytes, hepatocyte necrosis and infiltrates of mononuclear leukocytes. Occasional foci of hemorrhage were present. At the 1600 mg/kg concentration, proliferation of fibroblasts was observed. At 25-84

mg/kg, mild hydropic swelling and necrosis and moderate fatty changes were noted in the liver.

Test condition : Eight purebred beagle dogs (4/sex) actively immunized against distemper, infectious hepatitis, leptospirosis and rabies were acclimated for 7 days prior to treatment. They rats were housed individually and provided water ad libitum. A daily portion of Purina Laboratory Canine Diet was offered each day. Dogs were randomly allocated to 4 groups of 2/sex. The groups were given diet containing 25, 100, 400 or 1600 mg/kg/day test material for 30 consecutive days.

Test material was mixed with the diet using a Patterson-Kelley Twin Shell Blender at the appropriate concentrations based on the mean body weight of the dogs. A daily portion of 250 g/dog was offered each afternoon from days 0-21 and 300 g/dog from day 22-30.

All animals were observed daily to detect possible signs of toxicity. Special attention was placed on appearance, behavior and excretory functions. Food intake was recorded daily and body weight was measured prior to treatment, weekly during treatment and at termination. Necropsies were performed on all animals at study termination. At termination, all animals were euthanized and exsanguinated. Weights of the brain, gonads, heart, kidneys, liver, pituitary and thyroids were recorded and relative organ weights/body weight were calculated. The adrenal, aorta, gall bladder, urinary bladder, bone, bone marrow, cerebellum, cerebrum, colon, esophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, skeletal muscle, sciatic nerve, ovary, pancreas, parathyroid, pituitary, prostate, salivary gland, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea and uterus were preserved in buffered 10% formalin for possible future examination.

Test substance : The test material was Cyanox 1790. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

Conclusion : At 1600 mg/kg, reduced food consumption could have been due to the unappetizing nature of the diet. Necropsy findings and organ weight data indicated possible treatment-related effects on the liver at 400 and 1600 mg/kg.

Reliability : (4) not assignable
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. Clinical chemistry, urinalysis and hematological parameters were not measured. Only 2 animals were tested per dose. There were no negative controls. The amount of material given to dogs in the 25, 100 and 400 mg/kg/day groups was much higher than the desired doses from days 22-30.

24.05.2005 (25)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Bacterial reverse mutation assay
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2uvrA
Test concentration : 0, 62, 185, 556, 1667 and 5000 micrograms/plate (Test 1); 0, 125, 250, 500, 1000 and 2000 micrograms/plate (Test 2)
Cytotoxic concentr. : > 5000 micrograms/plate
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 471
Year : 2001
GLP : yes

Test substance : as prescribed by 1.1 - 1.4

Result : The test material was not positive in any of the strains tested in the absence and presence of S-9 mix. In test 1, the mean numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA were 31, 139, 14, 11 and 34 without S-9 and 35, 136, 16, 9 and 40 with S-9 (respectively). With the test material, the numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA ranged from 23-32, 103-135, 18-25, 5-9 and 26-37 without S-9 and 34-53, 111-150, 10-15, 6-11 and 21-34 with S-9 (respectively). In test 2, the mean numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA were 36, 164, 19, 19 and 32 without S-9 and 64, 156, 18, 24 and 40 with S-9 (respectively). With the test material, the numbers of revertants in negative control cultures of S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA ranged from 38-45, 160-175, 17-29, 15-22 and 29-36 without S-9 and 54-66, 163-172, 14-25, 20-24 and 36-49 with S-9 (respectively).

The test was valid, as the positive controls induced at least the minimum number of revertants as specified by the protocol. The test material was toxic at the 4 highest concentrations tested in Salmonella strain TA1537 in the first test, but not the second.

Test condition : Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were obtained from Dr. Bruce Ames (University of California, Berkeley, USA) and Escherichia coli WP2uvrA was obtained from Dr. C. Voogd, National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands. Frozen stocks of each strain were tested for histidine or tryptophan requirement and sensitivity to ampicillin, crystal violet and UV radiation (where appropriate).

S-9 was prepared from the livers of twelve male Wistar rats five days after they had been induced i.p. with a single dose of 500 mg/kg Aroclor 1254 in soya bean oil (20% w/v). The S-9 was assayed for sterility (0 colonies/10 microliters), protein content (32.1 g/l) and cytochrome P-450 content (29.6 micromoles/l; 0.924 micromoles/g protein).S-9 was frozen until use. On the day of use, aliquots of S-9 were thawed, mixed with a NADPH-generating system, and kept on ice until use.

The material was tested in two separate plate incorporation assays. Test material was dissolved in DMSO at 50 mg/l for the first assay and 20 mg/l for the second. A clear solution was obtained. This solution was used to make serial dilutions by 3-fold intervals in the first assay (62, 185, 556, 1667 and 500 micrograms/plate), and 2-fold intervals in the second assay (125, 250, 500, 1000, and 2000 micrograms/plate). In both assays, the 3 highest concentrations precipitated in the top agar. Positive controls were 1.0 microgram/plate sodium azide (strains TA100 and TA1535 without S-9), 80 micrograms/plate 9-aminoacridine (strain TA1537 without S-9), 2 micrograms/plate 2-nitrofluorene (strain TA98 without S-9), 100 micrograms/plate N-ethyl-N-nitrosourea (strain WP2uvrA without S-9), 2-aminoanthracene (2 micrograms/plate for strains TA98, TA100, TA1535 and 80 micrograms/plate for WP2uvrA), and bezo(a)pyrene (4 micrograms/plate for strain TA1537).

Bacteria (0.1 ml of a fully grown culture), test material, negative control (DMSO) or positive control (0.1 ml of the appropriate dilution), and 0.5 ml of sodium phosphate buffer (for tests without metabolic activation) or 0.5 ml of S-9 mix (for tests with metabolic activation) were mixed with 2 ml molten top agar (containing 0.6% agar, 0.5% NaCl and 0.05 mM L-histidine HCl/0.05 mM biotin for Salmonella and supplemented with 0.05 mM tryptophan for E coli), and the mix was poured onto minimal glucose agar

plates (1.5% agar in Vogel and Bonner medium E with 2% glucose). All plates were prepared in triplicate. The plates were incubated at 37 degrees C for 3 days. Subsequently, the his+ (Salmonella) and trp+ (E. coli) revertants were counted. The background lawn of bacteria growth was examined microscopically to determine if the material caused toxicity.

The study was considered valid if the mean colony counts of controls were within acceptable ranges and the positive controls caused a minimum 3-fold (strains TA100 or WP2uvrA without S-9 and TA98, TA100 and TA1537 with S-9), 5-fold (strain TA1535 with and without S-9 and WP2uvrA with S-9), or 10-fold (strain TA1537 without S-9) increase in the number of revertants. In addition, plates lost through contamination or other unforeseen events had to be < = 5%.

A response was considered positive if the mean number of revertants on the test plates was 2-fold greater than that of negative controls. A response was equivocal is the mean number of revertants was increased by 2-fold in strain TA100 or slightly less than 2-fold for the other strains.

A second test was to be conducted if the first test was inconclusive. The first test was considered to be inconclusive if < 5 analyzable concentrations were obtained, if a positive or equivocal response at only one concentration was observed or if positive or equivocal responses at several concentrations were observed that were not concentration-dependent.

A test material was considered to be mutagenic if a concentration-related increase or reproducible positive response was observed. A test material was not mutagenic if it produced neither a dose-related increase in the mean number of revertants nor a reproducible response at any of the time points. Both numerical significance and biological relevance were considered together in the evaluation.

Test substance : The purity of the test material was 93.7-98.9%.
Reliability : (1) valid without restriction
The test was performed according to an established guideline.
Flag : Critical study for SIDS endpoint
14.04.2005 (18)

Type : Mouse lymphoma assay
System of testing : cultured mouse lymphoma L5178Y cells
Test concentration : 6.2, 12.5, 25, 50, 100 and 200 micrograms/ml for test 1, 6.6, 8.2, 10, 13, 16, 20 and 25 micrograms/ml for test 2 without S-9 and 25, 33, 41, 51, 64, 80 and 100 micrograms/ml for test 2 with S-9
Cytotoxic concentr. : > = 20 micrograms/ml (without S-9), > = 51 micrograms/ml (with S-9)
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 476
Year : 2001
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Turbidity was present at 100 and 200 micrograms/ml in the first experiment (both in the presence and absence of S-9). Slight turbidity was observed at 100 micrograms/ml in the second experiment (in the presence of S-9).

Although the methods section stated that 0.1 mM methyl methanesulphonate (MMS) was used as a positive control in both tests without S-9, the results table for test 1 indicates that 10 mM MMS was used. It is believed that this is a misprint, since the number of mutants produced was similar to that produced in the second study with 0.1 mM MMS.

Result : There was no concentration-related increase in mutants in the presence or absence of S-9 in either study. In the presence of S-9 mix, the mutant

frequencies were increased at single concentrations of 100 micrograms/ml in the first test (414 x 10E6 mutants in treated vs. 889 and 109 x 10E6 mutants in the negative controls) and 33 micrograms/ml in the second (724 x 10E6 mutants in treated vs. 96 and 130 x 10E6 mutants in the negative controls). Both findings were not statistically significant. At these concentrations, more large (approximately 70%) than small colonies (approximately 30%) were formed. In the negative and positive controls incubated with S-9, the ratios of large:small colonies are approximately 60:40 and 50:50, respectively. In both assays, a Bartlett test was significant at $p < 0.05$, indicating the presence of an outlier. Therefore, the findings were not considered to be indicative of mutagenic activity.

In the absence of S-9 mix, a dose related decrease of the initial cell yield, relative suspension growth (RSG) and relative total growth (RTG) was observed at concentrations > 16 micrograms/ml. At the highest concentration tested (25 micrograms/ml), the RTG in the first assay was 6% (only one culture survived) and 36 and 75% in the second test. In the presence of S-9 mix, the RSG and RTG were affected at concentrations > 41 micrograms/ml. At the highest concentration tested in the first test (100 micrograms/ml), the RTG was 2 and 6%. In the second assay, the RTG was 31 at 26% at 80 micrograms/ml.

Test condition : The test was valid, since all requirements for validity were fulfilled. Mouse lymphoma L5178 cells (L5178Y tk +/- 3.2.7.c line) were obtained from Dr. J Cole, MRC Cell Mutation Unit, University of Sussex, UK. The chromosome number was 40 (stable aneuploid karyotype, 2n = 40). The cells were stored frozen in liquid nitrogen. Each new stock tested negative for mycoplasma contamination. Subcultures were prepared 5-7 days prior to experimental use. These cells (about 1 x 10E7/75 cm2 culture flask) were seeded in 50 ml RPMI 1640 medium (with HEPES and Glutamax-I) supplemented with 10% heat-inactivated horse serum, sodium pyruvate and penicillin/streptomycin. Cells were then incubated at 37 degrees C in humidified air containing 5% CO2. Fresh cells were harvested from a number of culture flasks and suspended in culture medium with 10% horse serum and counted. The growth rate (14.0 and 11.4 hr doubling time for tests 1 and 2) and viability of the cells (95% and 93% for each test) were checked on the day of exposure.

The test material was dissolved in DMSO (20 or 10 mg/ml for tests 1 and 2, respectively). Serial dilutions in DMSO were prepared from this clear stock solution, so that addition of 100 microliters of each to a final volume of 10 ml culture medium would produce the desired test concentrations (6.2, 12.5, 25, 50, 100 and 200 micrograms/ml for test 1, 6.6, 8.2, 10, 13, 16, 20 and 25 micrograms/ml for test 2 without S-9 and 25, 33, 41, 51, 64, 80 and 100 micrograms/ml for test 2 with S-9).

S-9 was prepared from the livers of twelve male Wistar rats five days after they had been induced i.p. with a single dose of 500 mg/kg Aroclor 1254 in soya bean oil (20% w/v). The S-9 was assayed for sterility (4 colonies/10 microliters), protein content (32.1 g/l) and cytochrome P-450 content (29.6 micromoles/l; 0.92 micromoles/g protein). S-9 was frozen until use. On the day of use, aliquots of S-9 were thawed, mixed with a NADPH-generating system, and kept on ice until use.

For the test without metabolic activation, 100 microliters of test material, negative control (1% DMSO) or positive control (0.1 mM methane methylsulphonate) and 4.9 ml culture medium (without horse serum) were added to 5 x 10E6 cells in 5 ml culture medium (with 10% horse serum) to a final volume of 10 ml. Duplicate cultures for each concentration and negative control and one positive control culture were prepared. Cells were incubated for 24 hr at 37 degrees C. At the beginning and end of the study, all cultures were checked visually and selected cultures were

checked for viability using trypan blue exclusion. For the test with metabolic activation, 100 microliters of test material, negative control (1% DMSO) or positive control (10 micrograms/ml 3-methylcholanthrene), 3.9 ml culture medium (without horse serum), and 1 ml 20% (v/v) S9-mix were added to 5 x 10E6 cells in 5 ml culture medium (with 10% horse serum) to a final volume of 10 ml. Duplicate cultures for each concentration and negative control and one positive control culture were prepared. They were incubated for 4 hours and checked for viability as described above.

The cytotoxicity of the test material was determined by counting the cells after exposure (see above) and by measuring the relative suspension growth (cumulative growth rate of cells 24 and 48 h after treatment compared with untreated controls) and relative total growth (product of the relative suspension growth and the cloning efficiency at 48 hours).

The frequency of trifluorothymidine (TFT) mutants and cloning efficiency (see below) of the cells were determined 48 hours after treatment. At this time, the cell suspensions were diluted to 10,000/ml in culture medium (with 20% horse serum) containing 4 micrograms TFT/ml. Aliquots (200 microliters) were transferred to each well of two 96-well microtiter plates, and the plates were incubated for 10-14 days at 37 degrees C. Four plates were prepared if a reduced cloning efficiency (< 50%) was expected. After the incubation period, the number of wells containing no growth was determined and the cloning efficiency in the TFT plates was calculated. The mutant frequency/1 x 10E6 clonable cells was then calculated. The numbers of colonies that were small (which are produced predominantly by chromosome rearrangements) and large (which are produced predominantly by point mutations) in the negative and positive controls and cultures treated with some of the test concentrations also were scored.

The cloning efficiency of the cells was calculated from the total number of negative wells on two microtiter plates and the number of cells seeded per well. To assess the cytotoxic effects of the test material or the positive controls on the cells, the initial cell yield after treatment, the relative suspension growth (at 24 and 48 hours) and the relative total growth (at 48 hours) compared to that of the negative vehicle controls were calculated. The cloning efficiencies of the cells with and without TFT were used together to calculate the mutant frequency, which was expressed as the number of TFT-resistant mutants per 1 x 10E6 clonable cells.

The tests were considered to be valid if the average cloning efficiency of the negative controls was >=60% and <=140% and fell within the range of 40-300 TFT-resistant mutants per 1 x 10E6 clonable cells, the mutant frequency of the positive controls was > 400 mutants/ 1 x 10E6 clonable cells and was at least twice that of the negative control, and the highest concentration used should cause cytotoxicity (unless the highest possible concentration that could be tested based on solubility, pH and osmolar effects was not cytotoxic).

Results at single concentrations were compared to values for negative controls using Dunnett's multiple comparison test. Concentration-related increases in mutant frequency were determined by linear regression and lack of fit. A response was considered to be positive if the p-value of the comparison between test and negative control values was < 0.05. Any apparent increase in mutant frequency at concentrations causing > 90% cytotoxicity was considered to be an artifact. A test material was considered mutagenic if a statistically significant concentration-related increase in the frequency of mutants occurred, or if a reproducible and statistically significant positive response for at least one of the test substance concentrations was observed. A material tested negative if it produced neither a statistically significant dose-related increase in the frequency of mutants nor a statistically significant and reproducible positive

response at any of the time points. Both numerical significance and biological relevance were considered together in the evaluation.

Test substance : The purity of the test material was 93.7-98.9%.
Reliability : (1) valid without restriction
The test was performed according to an established guideline.

14.04.2005 (24)

Type : Chromosomal aberration test
System of testing : Cultured Chinese Hamster Ovary Cells (CHO K-1 line)
Test concentration : 0.06 to 250 micrograms/ml (test 1); 12.5, 25, 50, 75, 125, and 250 micrograms/ml (test 2)
Cytotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 473
Year : 1991
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : For test 1 in the presence of S-9, the highest concentration tested (250 micrograms/ml) did not decrease the mitotic index. This concentration also caused a 36% decrease in the mitotic index after 4 hrs of incubation without S-9 (test 1), and 27% and 8% decreases in two experiments with S-9 (test 2). In these instances, this concentration did not cause the desired degree of inhibition (50-70%). Higher concentrations than 250 micrograms/ml could not be tested due to problems with solubility.

Result : In both assays, there was no significant effect of the test material on the number of cells with aberrations (with and without metabolic activation). In all experiments, the maximum percentage of cells treated with test material or vehicle that exhibited chromosome aberrations was 1.0%. In both assays, the negative control values were within historical ranges and the positive controls caused a significant increase in the number of cells with aberrations.

Test condition : The CHO cells (CHO K-1 line) were obtained from Dr. A. T. Natarajan, University of Leiden, The Netherlands. The chromosome number was 21-22 (stable aneuploid karyotype), and the cell cycle time was 12-14 hr. The cells were stored as frozen stock cultures in liquid nitrogen. Subcultures were prepared (passage 16) for experimental use. Cells were incubated at 37 degrees C in humidified air containing 5% CO2. At each passage, tests for mycoplasma contamination and karyotype stability were negative and stable, respectively. Cells were cultured in Ham's F-12 medium with Glutamax-I, supplemented with heat-inactivated fetal calf serum (10%), penicillin (100 IU/ml) and streptomycin (100 micrograms/ml).

S-9 was prepared from the livers of twelve male Wistar rats five days after they had been induced i.p. with a single dose of 500 mg/kg Aroclor 1254 in soya bean oil (20% w/v). The S-9 was assayed for sterility (4 colonies/10 microliters), protein content (32.1 g/l) and cytochrome P-450 content (29.6 micromoles/l; 0.92 micromoles/g protein). S-9 was frozen until use. On the day of use, aliquots of S-9 were thawed, mixed with a NADPH-generating system, and kept on ice until use.

The test material could not be dissolved in DMSO at 500 gm/ml (the concentration needed to yield the maximum concentration of 5 mg/ml stipulated by the protocol). The maximum concentration that could be dissolved in DMSO was 100 mg/ml. This concentration was sonicated for 3 min and was clear immediately after sonication (but not 30 minutes later). Serial dilutions from 0.2 - 100 mg/ml were prepared in DMSO, and 50 microliters of these solutions were added to culture medium (4.95 ml) to produce test concentrations ranging from 20 - 1000 micrograms/ml. Immediately after addition of the test material and throughout incubation at 37 degrees C for 18-24 hours, the medium was turbid and the test material

flocculated in the medium at 125 - 1000 micrograms/ml. Therefore, for the first test, the highest concentration chosen was 250 micrograms/ml.

For the first test, exponentially growing cells (120,000 cells/flask) were seeded in 25 cm2 tissue culture flasks containing 5 ml culture medium and incubated at 37 degrees C overnight. Cells were exposed to S-9 mix (0.5 ml) or an equivalent amount of culture medium, plus test (0.06, 0.125, 0.25, 0.5, 1. 2, 3.9, 7.8, 15.6, 31.3, 62.5, 125, and 250 micrograms/ml, negative control (DMSO) or positive control materials (0.1 and 0.025 micrograms/ml mitomycin C in the absence of S-9 and 3.75 micrograms/ml cyclophosphamide in the presence of S-9 mix) the next day. All treatments were done in duplicate. For the experiments with S-9, the culture medium did not contain fetal calf serum. The treatment time was 4 hr (pulse treatment) and 18 hr (continuous treatment) in the absence of S-9, and 4 hr (pulse treatment) and 4 hr only in the presence of S-9. Cells treated for only 4 hours were washed twice with phosphate-buffered saline and placed in fresh medium that did not contain test material after 4 hours. In both the absence and presence of S-9, the harvest time was 18 hr after treatment.

Cells were examined visually immediately after treatment, before the medium replacement at 4 hours, and 16 hours after treatment.

The conditions of the second test were identical to the first test with the following exceptions: the concentrations used were 12.5, 25, 50, 75, 125, and 250 micrograms/ml, the treatment/harvest times were 18/18 and 32/32 hr in the absence of S-9 mix and 4/18 and 4/32 hr in the presence of S-9 mix, and the positive control in the absence of S-9 mix was 0.05 micrograms/ml mitomycin C.

Two hours before incubations were terminated, cells were arrested in metaphase by adding colcemid (final concentration 0.1 mM). Cells were harvested at their designated time by trypsinization, treated for 15 min at 37 degrees C with a hypotonic solution (1% sodium citrate), fixed with a 3:1 mixture of methanol:glacial acetic acid, and transferred to slides (2/culture). Slides were stained in a 2% solution of Giemsa, rinsed in water, dried and covered with a coverslip. The slides were coded blindly. At least 1000 nuclei per culture were examined (500 per slide) to determine the mitotic index. In both assays, at the sampling times of 18 and 32 hours, the positive and negative controls and at least 3 concentrations of test material were selected for analysis of chromosomal aberrations [62.5, 125 and 250 micrograms/ml for test 1 with S-9 and the 4-hr test 1 without S-9 ; 15.6, 31.3 and 62.5 micrograms/ml for the 18-hr test 1 without S-9; 5, 10, and 20 micrograms/ml for test 2 without S-9 (18 and 32 hr); and 75, 125 and 250 micrograms/ml for test 2 with S-9 (both harvest times)]. If possible, the highest concentration analyzed reduced the mitotic index by 50-70% (compared to the negative control) or exhibited some other clear indication of toxicity.

For each treatment group, 200 well-spread metaphases per concentration (100 per culture), each containing 20-22 cetromeres were analyzed by microscopic examination for chromatid-type aberrations (gaps, breaks, fragments, interchanges), chromosome-type aberrations (gaps, breaks, minutes, rings, dicentrics) and other anomalies, such as interstitial deletions, endoreduplication, polyploidy and multiple aberrations (> 10/cell, excluding gaps), according to the criteria recommended by Savage (J Med Genet 13:103-122). Heavily damaged or endoreduplicated cells were recorded but not counted and included in the 200 analyzed cells. Gaps (achromatic lesions) were recorded separately and were not indicated in the final assessment). The Vernier readings of all aberrant metaphases were recorded.

Data from treated and control cultures were analyzed statistically by the

Fisher's exact probability test (2-sided). The study was considered valid if the positive controls gave a statistically significant increase in the number of aberrant cells and the negative controls were within the historical range.

A response was considered positive if the percentage of cells with structural chromosomal aberrations is statistically higher ($p < 0.05$) compared to the negative control. It was equivocal if the p value was > 0.05 but < 0.1. The material was considered to be clastogenic if a dose-related increase in the percentage of cells with structural aberrations was observed compared to control, or if a single positive result occurred in both tests. A test material was negative if there was neither a dose-dependent increase in aberrations nor a reproducible positive value in the tests. Both statistical significance and biological relevance were considered together in the evaluation.

Test substance : The purity of the test material was 93.7-98.9%.
Reliability : (1) valid without restriction
The test was performed according to an established guideline.

14.04.2005 (4)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	NMRI
Route of admin.	:	
Exposure period	:	
Doses	:	200, 1000 and 5000 mg/kg
Result	:	negative
Method	:	other
Year	:	1983
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Remark : It was not actually stated anywhere in the test that the material was given orally by gavage. However, based on the doses given this is assumed.

Result : One male mouse treated with 5000 mg/kg died after the second application of test material. There was no significant difference between the numbers of micronucleated polychromatic (PCE) or normochromatic erythrocytes (NCE) in treated animals versus vehicle-treated controls. The average numbers of PCE and NCE in control animals were 1.8 and 0.95, respectively. In the three groups of treated animals, the average numbers of PCE and NCE ranged from 1.2-2.0 and 0.6-1.4, respectively. There was no effect of treatment on the ratio of PCE to NCE (ranged from 0.2-2.2 in control and 0.8-2.1 in treated). The test was valid, as there was a significant increase in the number of micronucleated erythrocytes in the positive control group (23-46 micronucleated PCE/animal and 6-14 micronucleated NCE/animal in positive controls vs. 0-3 micronucleated PCE and 0-2 micronucleated NCE/animal in the negative control).

Test condition : Sixty-eight NMRI KFM mice (34/sex, 5 weeks old, 20-39 g) were acclimated for 13 days before being used in the test. They were clinically examined by a veterinarian during this period and did not have any symptoms of disease. Mice were randomized into 3 treatment groups of 6 animals/sex (200, 1000 and 5000 mg/kg), one positive control group of 6 animals/sex (50 mg/kg cyclophosphamide) and one negative control group of 8 animals/sex [(Aqua Bidest with Tween 80(1%)]. For each dose of test material, a suspension was prepared by adding the material to Aqua Bidest containing 1% Tween 80. The suspension was homogenized and stirred with a magnetic stir bar while animals were being dosed. The test and control materials were administered by gavage on two consecutive days in

a volume of 20 ml/kg. Animals received pelleted standard Kliba 343-QA mouse maintenance diet and water ad libitum before and during the test.

All animals were killed by cervical dislocation 24 hours after the last treatment (48 hours after the first treatment). Both femurs were removed from each mouse and freed of adherent tissue. Bone marrow cells were flushed out of the femurs using a needle and syringe containing 0.2 ml calf serum. Cells from both femurs were centrifuged at 1000 rpm for 5 min. Cells in the sediment were carefully mixed by aspiration in a siliconized Pasteur Pipette. A small drop of the suspension was smeared onto a slide and slides were air dried overnight. Two slides were prepared per animal. The following day, the slides were stained according to the Panoptic staining method of Pappenheim (as described in Queisser, Das Knochenmark, Georg Thieme Verlag, Stuttgart 1978, pg 12).

Slides from each animal (with the exception of 1 animal/sex from each treatment group) were blindly evaluated for the presence of micronuclei. One thousand polychromatic erythrocytes (PCE) and one thousand normochromatic erythrocytes (NCE) from each slide were screened under a microscope (at 1000x). The ratio of polychromatic to normochromatic erythrocytes based on 150 PCE and NCE per slide was calculated. The ratio of PCE/NCE was calculated and used as an index of toxicity of the test material.

Homogeneity of test results was confirmed using the Poisson Heterogenicity test. The results of the positive control tests were not included in the analysis since they were so much higher than those of the test material. The 95% confidence limits for the L parameter of the Poisson distributions were taken from Geigy tables. Since results did not appear to be dependent on dose of the material, all doses of the material per sex were analyzed globally (compared to the negative control) using regression. The test material was considered positive if the T value (one-sided) was significant.

Test substance : The stated purity of the material was > 95%.
Reliability : (1) valid without restriction
The study is comparable to a guideline study.
Flag : Critical study for SIDS endpoint
14.04.2005 (15)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : other: combined reproductive/developmental toxicity screening test
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period :
Frequency of treatm. : continuous
Premating exposure period
Male : 14 days
Female : 14 days
Duration of test : to postpartum day 5
No. of generation studies : 1
Doses : 1000, 10000 and 20000 ppm
Control group : yes

NOAEL parental	:	= 1000 ppm
other: NOAEL fertility	:	= 10000 ppm
other: NOAEL developmental	:	= 20000 ppm
Method	:	OECD Guide-line 421
Year	:	2005
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Remark : Doses of 1000, 10000 and 20000 ppm are equivalent to 69, 651 and 1294 mg/kg/day in males and 77, 782 and 1558 mg/kg/day in females.

Increased post-implantation loss and the resultant decrease in litter size at 20000 ppm are likely to be consequences of maternal toxicity and/or stress from exposure to the irritating test material. The effects of maternal toxicity on embryofetal survival and development have been reviewed by Khera (Teratology 31: 129-153, 1985). Khera et al. found a "fairly strong" association between embryo-fetal mortality (post-implantation loss) and maternal toxicity (significant reduction in weight gain, test agent-related pharmacologic or toxicologic signs of behavior, abortion or death) in an analysis of data from studies in hamsters, mice, rats and rabbits. Embryo-fetal deaths and maternal toxicity were co-reported in 133/177 (75%) of the studies and embryo-fetal deaths at doses causing no apparent maternal toxicity were reported in only 9/140 (6%) of the studies.

Result : Mortality and Clinical Signs: There were no treatment-related mortalities. One control female was killed in extremis due to an injury to the tail. No other macroscopic abnormalities were observed at the post mortem examination (with the exception of the skin lesions noted below).

At 20000 ppm, three males and all females showed scabs and fur loss on the top of the head and/or around the snout from Day 22 of treatment onwards. These findings were noted in all females, but in no males treated with 10000 ppm. For all but one 10000 ppm female, these signs persisted until completion of the study. Scabbing also was noted on the abdomens of two females treated with 20000 ppm. The scabbing was considered to be an adverse effect of treatment due to its severity. There were no clinical signs of toxicity or irritancy in animals treated with 1000 ppm. One control female had approximately 2 inches of skin missing from the end of the tail, exposing underlying tissue.

Body weight and food consumption: There was no significant effect of treatment on body weight or body weight gain during maturation, gestation or lactation. There was a statistically significant ($p<0.05$) increase in the amount of food eaten during the first week of treatment in male rats treated with 20000 and 10000 ppm compared to controls (31 g/rat/day in each group vs. 29 g/rat/day in control). This was considered to be incidental due to the lack of a dose-related response and comparable changes in female food consumption. Daily visual inspection of water bottles revealed no intergroup differences in water consumption.

Fertility: There were no significant treatment-related effects on mating performance or fertility. The majority of mating pairs had a precoital interval of four days or less. There were no treatment-related effects on gestation length. The majority of females showing positive evidence of mating gave birth to live young following 22 to 23 days of gestation. At 20000 ppm, no evidence of mating was observed for one mating pair but a live litter was produced. At 10000 ppm, one female was found to be not pregnant following positive evidence of mating. Another female was found to have a total litter loss in utero. At 1000 ppm, all females produced a live litter. One control pair failed to mate within the fourteen day mating phase.

There was no significant effect of treatment on the total number of

implantation sites (ranged from 13 +/- 5 in mid dose group to 17 +/- 2 in low dose group, number of corpora lutea (ranged from 14 +/- 5 in mid dose group to 18 +/- 3 in low dose group), or pre-implantation loss (ranged from 4.4 +/- 5.7% in mid dose group to 8.0 +/- 7.7% in control). At 20000 ppm, post-implantation losses (17.7 +/- 18.7%) were increased with respect to control (5.4 +/- 9.9%). This increase was not statistically significant. Study personnel considered this finding to be of toxicological significance, since there were four individuals with higher post-implantation loss values than the highest control value of 28.6%. At 10000 ppm, post-implantation losses (21.0 +/- 32.7%) were also increased compared to controls (not significant). This was predominantly due to one female with a total litter loss in utero. This value was a statistical outlier and not representative of the group. Therefore it should be considered a chance event. Without the female with total litter loss, the post-implantation loss in the 10000 ppm group was 11.1 +/- 14.9%). There was no effect of treatment with 1000 ppm on post-implantation loss (3.5 +/- 5.5% vs. 5.4 +/- 9.9% in control).

Organ weight, gross and histopathology data: There was no effect of treatment on parental organ weight. At 20000 ppm, fur loss and scabbing was observed in all females and 3 males (predominantly on the head). Similar skin lesions were noted on the abdomens of two females at this dose level. Fur loss and scabbing on the head also were observed in 9 females treated with 10000 ppm. At 1000 ppm, one male showed sloughing of the glandular region of the gastric epithelium. This was not considered to be related to treatment due to the lack of similar findings at higher doses. One control female had approximately two inches of skin missing from the tip of the tail exposing underlying tissue.

Acanthosis, occasionally with focal epithelial ulceration and overlying scab formation, was observed in 9/10 females dosed with 20000 ppm ($p<0.001$) and 8/9 females dosed with 10000 ppm ($p<0.01$). One male at 20000 ppm also was affected. All remaining morphological changes were similar in incidence and degree of severity to controls and were commonly observed in laboratory maintained rats of the same age and strain. Specifically, no significant histopathological abnormalities were observed in the gonads or other accessory sex organs of control or treated rats.

Offspring data: There were no statistically significant effects of treatment on live birth or viability index (ranged from 97% in high dose to 100% in control and from 91% in control to 100% in high dose, respectively), litter size (ranged from 11.2 +/- 3.4 in high dose to 16.0 +/- 1.9 in low dose at postpartum day 1 and from 11.2 +/- 3.4 in high dose to 15.4 +/- 1.3 in mid dose at post parturm day 4), pinna unfolding, surface righting reflex or sex ratio. Although group mean litter size was not statistically lower than control in treated animals, the number of females with litters of 10 or fewer offspring was higher than control (3 vs. 1). There were no significant treatment-related gross abnormalities in offspring at termination.

There was no significant effect of treatment on litter weight at Days 1 and 4 postpartum (although weights decreased in a dose-dependent manner). Average weights of litters at Day 1 postpartum were 92.2, 96.7, 88.6 and 74.1 grams in the control, 1000 ppm, 10000 ppm and 20000 ppm groups, respectively. Average weights of litters at Day 4 were 118.9, 128.0, 119.2 and 109.5 grams in the same groups. This is probably due to the tendency towards a lower number of animals/litter in the mid and high dose groups. Average weights of pups at days 1 and 4 did not differ between groups (ranged from 6.1 in the low dose to 7.0 grams in the mid dose group and from 8.3 in the low dose group to 10.0 grams in the high dose group, respectively).

Test condition : Date of study: June 18, 2004 - February 11, 2005

Animals: Male and female Sprague-Dawley Crl:CD® (SD)IGS BR rats were

obtained from Charles River (UK) Limited, Manston Road, Margate, Kent. They were examined on the day of receipt and acclimated for 7 days. A total of 40 animals/sex were accepted into the study. The males and females weighed 340-394 g and 209-243 g at the start of the study, respectively.

Animals were housed in groups of five by sex in polypropylene cages with stainless steel grid floors and tops, suspended over paper-line polypropylene trays (except during mating and gestation). One male and one female were housed in similar cages during mating. After evidence of successful mating, males were returned to their original cages. The females were then housed individually in cages with solid floors, soft wood chip bedding and stainless steel tops.

Animals were housed in an environmentally-controlled room, under a 12 hour light/dark cycle. The temperature and relative humidity were maintained to operate within a target range of 21 +/- 2 degrees C and 55 +/- 15 %. Air was changed at least 15 times per hour. Certified feed and water were supplied ad libitum. No contaminants were present in food and water at levels sufficient to affect the outcome of the study.

Test material: The test material was incorporated into the diet at concentrations of 1000, 10000 and 20000 ppm by mixing a known amount of material with a known amount of basal laboratory diet for 20 minutes at a constant speed in a Hobart mixer. Samples were analyzed for stability and homogeneity by high performance liquid chromatography (with an external standard) before the beginning of the study. Samples for homogeneity analyses were taken from the middle and two opposite sides of the container, in triplicate. Samples for stability analyses were sampled and analyzed initially and after storage at ambient temperature in the dark for 14 days. Results of the analyses showed that the material was homogeneous in the diet and stable for at least 14 days. Dietary admixtures were prepared prior to treatment and weekly thereafter. Samples of each admixture were taken every 14 days and analyzed for concentration of test material. All admixutures were within acceptable limits of the desired concentration.

Study design: The animals were randomly allocated by weight to 4 groups of 10 animals per sex (F0 animals) receiving either 0 (vehicle), 1000, 10000 or 20000 mg/kg/day test material. Animals in each group were uniquely identified. Test material administration to F0 males and females began 14 days prior to mating and continued through mating. Females continued receiving test material to postnatal day 5.

Following 14 days of dosing, 10 F0 males were randomly paired on a 1:1 basis with 10 F0 females from the same group for up to 14 days. Following positive identification of mating (presence of a copulatory plug in the vagina or on the tray beneath the cage or the presence of sperm in a vaginal smear following vaginal lavage), the males were returned to their cages. Following mating, all F0 females were allowed to deliver naturally and rear their young to postnatal day 5 (the scheduled day of necropsy). During the period of expected parturition, the females were observed three times daily for parturition (twice on weekends). The date of mating, date and time of start and completion of parturition and duration of gestation were recorded for each female. After parturition was complete, the numbers of live and dead offspring were recorded. The following were recorded for each litter: number of pups born, number and sex of pups alive from days 1 to 4 postpartum, clinical condition of pups from birth to day 4 post partum and individual litter weights on days 1 and 4 postpartum. All live offspring were observed for surface righting reflex and detachment of pinna on day 1 post partum.

All F0 animals were observed twice daily for mortality and morbundity (once daily on weekends). Clinical observations were recorded daily. Male body weights and food consumption per cage were recorded weekly throughout the study. Body weights and food consumption per cage of females were recorded weekly until mating. After mating, body weights of females were recorded on gestation days 0, 7, 14, and 20 and on lactation days 1 and 4, and food consumption of females was determined for gestation days 1-7, 7-14 and 14-20. Female food consumption also was recorded for the period covering lactation days 1-4.

Males were euthanized after confirmation of successful mating. All surviving adults (including non-fertile animals) and offspring were euthanized on day 5 postpartum. All animals were examined microscopically for internal and external abnormalities. The numbers of corpora lutea and uterine implantation sites in all females were counted. The procedure was enhanced by staining the uteri with 1% ammonium polysulphide solution. The testes and epididymides of all adult males were weighed and preserved in Bouins solution. The coagulating glands, epididymides, prostate, seminal vesicles, testes, pituitary, ovaries, uterus/cervix, vagina and skin (from the top of the head) from the high dose and control adult males and females were fixed, processed and examined microscopically by a pathologist.

Statistical evaluations: Data were processed to give group mean values and standard deviations. The pre coital interval (time between initial pairing and evidence of mating), mating index, pregnancy index, gestation length, parturition index, live birth index, viability index and sex ratio were determined. A continuity correction of a half day was subtracted from the age of appearance of pinna detachment for those litters born overnight. Adult body weight, body weight gain and food consumption, litter size and weight, individual pup body weight, pinna detachment, reproductive and viability indices and organ weight data were analyzed for homogeneity using Bartlett's' test, followed by a one-way analysis of variance (ANOVA). Data that were not homogeneous were subsequently analyzed using a t-test (assuming unequal variances). Dunnett's multiple comparison method was used to analyze data that were homogenous. Individual pre-coital intervals, gestation length, offspring reflexological responses, sex ratios, pre- and post-implantation losses and relative organ weights were analyzed using the Kruskal-Wallis non parametric rank sum test. Post-implantation losses in the 10000 ppm group were also compared using Grubbs' test (a parametric test to determine outliers). Histopathological lesions that occurred at an overall frequency of 1 or greater were analyzed using a chi-squared test. Severity grades were analyzed using a Kruskal-Wallis one-way non-parametric ANOVA. Significant differences were reported at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ level (if present).

Test substance : The test material contained 96-97% 1,3,5-Tris[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-xylyl]methyl]1,3,5-triazine-2,4,6(1H,3H,5H)-trione (CAS No. 40601-76-1). Impurities were not listed.

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

24.05.2005 (17)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex :
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period :

Frequency of treatm.	:	continuous
Duration of test	:	14 days prior to mating to lactation day 5
Doses	:	1000, 10000 and 20000 ppm
Control group	:	yes
NOAEL maternal tox.	:	= 1000 ppm
NOAEL teratogen.	:	= 20000 ppm
other: NOAEL developmental	:	= 20000 ppm
Result	:	The material is not a developmental toxicant at the doses tested.
Method	:	other: OECD Guideline 421
Year	:	2005
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Remark : From this point forward, this summary is identical to the summary listed above under Section 5.8.1 (Toxicity to Fertility).

Doses of 1000, 10000 and 20000 ppm are equivalent to 69, 651 and 1294 mg/kg/day in males and 77, 782 and 1558 mg/kg/day in females.

Increased post-implantation loss and the resultant decrease in litter size at 20000 ppm are likely to be consequences of maternal toxicity and/or stress from exposure to the irritating test material. The effects of maternal toxicity on embryofetal survival and development have been reviewed by Khera (Teratology 31: 129-153, 1985). Khera et al. found a "fairly strong" association between embryo-fetal mortality (post-implantation loss) and maternal toxicity (significant reduction in weight gain, test agent-related pharmacologic or toxicologic signs of behavior, abortion or death) in an analysis of data from studies in hamsters, mice, rats and rabbits. Embryo-fetal deaths and maternal toxicity were co-reported in 133/177 (75%) of the studies and embryo-fetal deaths at doses causing no apparent maternal toxicity were reported in only 9/140 (6%) of the studies.

Result : Mortality and Clinical Signs: There were no treatment-related mortalities. One control female was killed in extremis due to an injury to the tail. No other macroscopic abnormalities were observed at the post mortem examination (with the exception of the skin lesions noted below).

At 20000 ppm, three males and all females showed scabs and fur loss on the top of the head and/or around the snout from Day 22 of treatment onwards. These findings were noted in all females, but in no males treated with 10000 ppm. For all but one 10000 ppm female, these signs persisted until completion of the study. Scabbing also was noted on the abdomens of two females treated with 20000 ppm. The scabbing was considered to be an adverse effect of treatment due to its severity. There were no clinical signs of toxicity or irritancy in animals treated with 1000 ppm. One control female had approximately 2 inches of skin missing from the end of the tail, exposing underlying tissue.

Body weight and food consumption: There was no significant effect of treatment on body weight or body weight gain during maturation, gestation or lactation. There was a statistically significant ($p<0.05$) increase in the amount of food eaten during the first week of treatment in male rats treated with 20000 and 10000 ppm compared to controls (31 g/rat/day in each group vs. 29 g/rat/day in control). This was considered to be incidental due to the lack of a dose-related response and comparable changes in female food consumption. Daily visual inspection of water bottles revealed no intergroup differences in water consumption.

Fertility: There were no significant treatment-related effects on mating performance or fertility. The majority of mating pairs had a precoital interval of four days or less. There were no treatment-related effects on gestation length. The majority of females showing positive evidence of

mating gave birth to live young following 22 to 23 days of gestation. At 20000 ppm, no evidence of mating was observed for one mating pair but a live litter was produced. At 10000 ppm, one female was found to be not pregnant following positive evidence of mating. Another female was found to have a total litter loss in utero. At 1000 ppm, all females produced a live litter. One control pair failed to mate within the fourteen day mating phase.

There was no significant effect of treatment on the total number of implantation sites (ranged from 13 +/- 5 in mid dose group to 17 +/- 2 in low dose group, number of corpora lutea (ranged from 14 +/- 5 in mid dose group to 18 +/- 3 in low dose group), or pre-implantation loss (ranged from 4.4 +/- 5.7% in mid dose group to 8.0 +/- 7.7% in control). At 20000 ppm, post-implantation losses (17.7 +/- 18.7%) were increased with respect to control (5.4 +/- 9.9%). This increase was not statistically significant. Study personnel considered this finding to be of toxicological significance, since there were four individuals with higher post-implantation loss values than the highest control value of 28.6%. At 10000 ppm, post-implantation losses (21.0 +/- 32.7%) were also increased compared to controls (not significant). This was predominantly due to one female with a total litter loss in utero. This value was a statistical outlier and not representative of the group. Therefore it should be considered a chance event. Without the female with total litter loss, the post-implantation loss in the 10000 ppm group was 11.1 +/- 14.9%). There was no effect of treatment with 1000 ppm on post-implantation loss (3.5 +/- 5.5% vs. 5.4 +/- 9.9% in control).

Organ weight, gross and histopathology data: There was no effect of treatment on parental organ weight. At 20000 ppm, fur loss and scabbing was observed in all females and 3 males (predominantly on the head). Similar skin lesions were noted on the abdomens of two females at this dose level. Fur loss and scabbing on the head also were observed in 9 females treated with 10000 ppm. At 1000 ppm, one male showed sloughing of the glandular region of the gastric epithelium. This was not considered to be related to treatment due to the lack of similar findings at higher doses. One control female had approximately two inches of skin missing from the tip of the tail exposing underlying tissue.

Acanthosis, occasionally with focal epithelial ulceration and overlying scab formation, was observed in 9/10 females dosed with 20000 ppm ($p<0.001$) and 8/9 females dosed with 10000 ppm ($p<0.01$). One male at 20000 ppm also was affected. All remaining morphological changes were similar in incidence and degree of severity to controls and were commonly observed in laboratory maintained rats of the same age and strain. Specifically, no significant histopathological abnormalities were observed in the gonads or other accessory sex organs of control or treated rats.

Offspring data: There were no statistically significant effects of treatment on live birth or viability index (ranged from 97% in high dose to 100% in control and from 91% in control to 100% in high dose, respectively), litter size (ranged from 11.2 +/- 3.4 in high dose to 16.0 +/- 1.9 in low dose at postpartum day 1 and from 11.2 +/- 3.4 in high dose to 15.4 +/- 1.3 in mid dose at post parturm day 4), pinna unfolding, surface righting reflex or sex ratio. Although group mean litter size was not statistically lower than control in treated animals, the number of females with litters of 10 or fewer offspring was higher than control (3 vs. 1). There were no significant treatment-related gross abnormalities in offspring at termination.

There was no significant effect of treatment on litter weight at Days 1 and 4 postpartum (although weights decreased in a dose-dependent manner). Average weights of litters at Day 1 postpartum were 92.2, 96.7, 88.6 and 74.1 grams in the control, 1000 ppm, 10000 ppm and 20000 ppm groups, respectively. Average weights of litters at Day 4 were 118.9, 128.0, 119.2 and 109.5 grams in the same groups. This is probably due to the tendency

towards a lower number of animals/litter in the mid and high dose groups. Average weights of pups at days 1 and 4 did not differ between groups (ranged from 6.1 in the low dose to 7.0 grams in the mid dose group and from 8.3 in the low dose group to 10.0 grams in the high dose group, respectively).

Test condition : Date of study: June 18, 2004 - February 11, 2005

Animals: Male and female Sprague-Dawley Crl:CD® (SD)IGS BR rats were obtained from Charles River (UK) Limited, Manston Road, Margate, Kent. They were examined on the day of receipt and acclimated for 7 days. A total of 40 animals/sex were accepted into the study. The males and females weighed 340-394 g and 209-243 g at the start of the study, respectively.

Animals were housed in groups of five by sex in polypropylene cages with stainless steel grid floors and tops, suspended over paper-line polypropylene trays (except during mating and gestation). One male and one female were housed in similar cages during mating. After evidence of successful mating, males were returned to their original cages. The females were then housed individually in cages with solid floors, soft wood chip bedding and stainless steel tops.

Animals were housed in an environmentally-controlled room, under a 12 hour light/dark cycle. The temperature and relative humidity were maintained to operate within a target range of 21 +/- 2 degrees C and 55 +/- 15 %. Air was changed at least 15 times per hour. Certified feed and water were supplied ad libitum. No contaminants were present in food and water at levels sufficient to affect the outcome of the study.

Test material: The test material was incorporated into the diet at concentrations of 1000, 10000 and 20000 ppm by mixing a known amount of material with a known amount of basal laboratory diet for 20 minutes at a constant speed in a Hobart mixer. Samples were analyzed for stability and homogeneity by high performance liquid chromatography (with an external standard) before the beginning of the study. Samples for homogeneity analyses were taken from the middle and two opposite sides of the container, in triplicate. Samples for stability analyses were sampled and analyzed initially and after storage at ambient temperature in the dark for 14 days. Results of the analyses showed that the material was homogeneous in the diet and stable for at least 14 days. Dietary admixtures were prepared prior to treatment and weekly thereafter. Samples of each admixture were taken every 14 days and analyzed for concentration of test material. All admixutures were within acceptable limits of the desired concentration.

Study design: The animals were randomly allocated by weight to 4 groups of 10 animals per sex (F0 animals) receiving either 0 (vehicle), 1000, 10000 or 20000 mg/kg/day test material. Animals in each group were uniquely identified. Test material administration to F0 males and females began 14 days prior to mating and continued through mating. Females continued receiving test material to postnatal day 5.

Following 14 days of dosing, 10 F0 males were randomly paired on a 1:1 basis with 10 F0 females from the same group for up to 14 days. Following positive identification of mating (presence of a copulatory plug in the vagina or on the tray beneath the cage or the presence of sperm in a vaginal smear following vaginal lavage), the males were returned to their cages. Following mating, all F0 females were allowed to deliver naturally and rear their young to postnatal day 5 (the scheduled day of necropsy). During the period of expected parturition, the females were observed three times daily for parturition (twice on weekends). The date of mating, date and time of start and completion of parturition and duration of gestation were recorded

for each female. After parturition was complete, the numbers of live and dead offspring were recorded. The following were recorded for each litter: number of pups born, number and sex of pups alive from days 1 to 4 postpartum, clinical condition of pups from birth to day 4 post partum and individual litter weights on days 1 and 4 postpartum. All live offspring were observed for surface righting reflex and detachment of pinna on day 1 post partum.

All F0 animals were observed twice daily for mortality and morbundity (once daily on weekends). Clinical observations were recorded daily. Male body weights and food consumption per cage were recorded weekly throughout the study. Body weights and food consumption per cage of females were recorded weekly until mating. After mating, body weights of females were recorded on gestation days 0, 7, 14, and 20 and on lactation days 1 and 4, and food consumption of females was determined for gestation days 1-7, 7-14 and 14-20. Female food consumption also was recorded for the period covering lactation days 1-4.

Males were euthanized after confirmation of successful mating. All surviving adults (including non-fertile animals) and offspring were euthanized on day 5 postpartum. All animals were examined microscopically for internal and external abnormalities. The numbers of corpora lutea and uterine implantation sites in all females were counted. The procedure was enhanced by staining the uteri with 1% ammonium polysulphide solution. The testes and epididymides of all adult males were weighed and preserved in Bouins solution. The coagulating glands, epididymides, prostate, seminal vesicles, testes, pituitary, ovaries, uterus/cervix, vagina and skin (from the top of the head) from the high dose and control adult males and females were fixed, processed and examined microscopically by a pathologist.

Statistical evaluations: Data were processed to give group mean values and standard deviations. The pre coital interval (time between initial pairing and evidence of mating), mating index, pregnancy index, gestation length, parturition index, live birth index, viability index and sex ratio were determined. A continuity correction of a half day was subtracted from the age of appearance of pinna detachment for those litters born overnight. Adult body weight, body weight gain and food consumption, litter size and weight, individual pup body weight, pinna detachment, reproductive and viability indices and organ weight data were analyzed for homogeneity using Bartlett's' test, followed by a one-way analysis of variance (ANOVA). Data that were not homogeneous were subsequently analyzed using a t-test (assuming unequal variances). Dunnett's multiple comparison method was used to analyze data that were homogenous. Individual pre-coital intervals, gestation length, offspring reflexological responses, sex ratios, pre- and post-implantation losses and relative organ weights were analyzed using the Kruskal-Wallis non parametric rank sum test. Post-implantation losses in the 10000 ppm group were also compared using Grubbs' test (a parametric test to determine outliers). Histopathological lesions that occurred at an overall frequency of 1 or greater were analyzed using a chi-squared test. Severity grades were analyzed using a Kruskal-Wallis one-way non-parametric ANOVA. Significant differences were reported at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ level (if present).

Test substance : The test material contained 96-97% 1,3,5-Tris[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-xylyl]methyl]1,3,5-triazine-2,4,6(1H,3H,5H)-trione (CAS No. 40601-76-1). Impurities were not listed.

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

24.05.2005 (17)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type	:	other:examination of reproductive organs from 90-day study
In vitro/in vivo	:	In vivo
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	continuous
Duration of test	:	90 days
Doses	:	25, 100 and 400 mg/kg bw
Control group	:	yes
Result	:	test material was not toxic to reproductive organs at the doses tested
Method	:	other
Year	:	1977
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	This study is described in more detail in Section 5.4.
Result	:	There was no effect of treatment on any of the organs examined.
Test condition	:	COBS Sprague-Dawley rats (80 per sex) were acclimated for 8 days before treatment. They were then assigned to 4 test groups [0 mg/kg bw (control diet), or 25, 100 or 400 mg/kg bw test material] of 20 per sex per group by weight stratification. At time of assignment, all animals were 4 weeks old. Weights of male and female animals ranged from 69-100 g and 64-95 g, respectively. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-75 degrees F and 40-50% relative humidity, with 10 exchanges of air/hour. The light cycle was 12 hr light/12 hours dark. Test diets were prepared by mixing the appropriate amount of test material with equal amounts of ground Purina Lab Chow in a high speed blender. After blending, an additional 2 parts of ground lab chow was mixed by hand into the blended material. After this step, an additional amount of ground lab chow was added to give the appropriate dose level (25 mg/kg bw, 100 mg/kg bw or 400 mg/kg bw). The diet was then placed in a twin-shell mixer and mixed until the test material was distributed throughout the diet. The formula used to determine the amount of material added to feed was as follows: mean body weight x dietary level x 7 days/mean weekly food consumption = mg test material/kg feed. Fresh diets were prepared at least once per week. Dosages were adjusted weekly on the basis of the weight and food consumption for the preceding week. Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. Clinical chemistries, hematologies and urinalyses were performed on selected animals. Organ weights (brain, gonads, heart, kidneys and liver) were measured in all animals that survived to day 90. At study termination, all animals were weighed. They were then euthanized. The urogenital orifaces were examined visually and by palpation for distortions. All subcutaneous tissues were examined including mammary glands. Abdominal contents were examined grossly. The following reproductive tissues were taken and preserved for possible histologic evaluation: prostate, testes, uterus, ovary and seminal vesicle. Any organ or tissue exhibiting a lesion was noted and the lesion was taken for histopathological examination. Complete histopathology was performed on 10 animals/sex/group from the control and high dose groups. These animals were selected randomly from a table.
Test substance	:	The purity of the test material was 90.0%. Impurities were not listed.

Reliability	:	(2) valid with restrictions Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. The effects on mating and offspring were not tested.
14.04.2005		(21)
Type	:	other: examination of organs from 90 day study
In vitro/in vivo	:	In vivo
Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	oral feed
Exposure period	:	91 days
Frequency of treatm.	:	1 hr/day
Duration of test	:	91 days
Doses	:	46 mg/kg/day
Control group	:	yes, concurrent no treatment
Result	:	no effect on the reproductive organs examined
Method	:	other
Year	:	1983
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Additional information from this study is located in Section 5.4.
Result	:	There was no effect of treatment on ovary or testes weights. There also was no effect of treatment on histology of any reproductive organ examined.
Test condition	:	Purebred beagle dogs (31/sex, approximately 5 months old) were acclimated for 55 days before use. The supplier had immunized the dogs against canine distemper, infectious canine hepatitis, canine leptospirosis, parovirus and rabies. A veterinarian concluded that they were healthy prior to shipment. The body weight range of the animals just prior to treatment was 5.6 to 9.3 kg for males and 4.5 to 8.5 kg for females. The dogs were housed individually in a room maintained under a 12 hr light/12 hr dark cycle and 12 air changes/hour. During the acclimation period, the animals were randomly assigned to 7 treatment groups of 4 animals/sex. Five of these groups were going to be treated with a compound B (which was not identified). One group served as a control group, and the other was to be treated with CL226,034 (Cyanox 1790). Diets containing the test material were prepared weekly by adding the required weight of the test material to known amounts of powdered certified dog chow and mixing for approximately 30 minutes in a Hobart mixer. Homogeneity and adequacy of mixing of the test diets were evaluated by analyzing samples from the top, middle and bottom of the mixer. Stability of the test diet was analyzed immediately after preparation and storage at ambient temperature for 7 and 14 days. Fresh diets were stored at approximately 4 degrees C in labeled airtight plastic containers for the period of use. The concentration of test material in the diets was checked during weeks 1, 2, 4, 8 and 13, immediately after preparation. From study day -5.1 to 0.1, the dogs were fed 400 g/day of a standard certified commercial dog food. From study day 0.2 to termination, the males were fed 450 g/day and the females 400 g/day. For one hour per day, test animals were fed diet containing test material (compound B or 46 mg/kg/day CL226,034). Fresh water was available ad libitum. There were no contaminants in the food or water that were expected to affect the outcome of the test. All controls and animals treated with CL226,034 were on test for a minimum of 91 days.

A complete gross necropsy was performed on each dog that survived to study termination. The animals were euthanized and exsanguinated, and the ovaries and testes were dissected and weighed. The ovaries, testes, epididymides, prostate, uterus (horns and body), and vagina were examined histologically.

Test substance : The test material was CL 226, 034, which is Cyanox 1790. It was supplied as a white powder from American Cyanamid. Documentation of purity did not accompany the study. According to a Cytec Industries Inc. (formerly known as American Cyanamid) MSDS of the material written in 2001, the purity of the material is 93.7 - 98.9%. Impurities are not listed on the MSDS.

Reliability : (2) valid with restrictions
The effects on mating and offspring were not tested. Only one dose was tested.

14.04.2005 (23)

Type : other:examination of organs from 90 day study
In vitro/in vivo : In vivo
Species : dog
Sex : male/female
Strain : Beagle
Route of admin. : oral feed
Exposure period : 90 days
Frequency of treatm. : continuous
Duration of test : 90 days
Doses : 7.5, 15, 30 mg/kg bw
Control group : yes, concurrent no treatment
Result : not toxic to the reproductive organs examined
Method : other
Year : 1977
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : This study is described in more detail in Section 5.4.
Result : There was no effect of treatment on the gross or histopathology of any organ examined.
Test condition : Thirty two pure bred Beagle dogs (16 per sex) were acclimated for 16 days before treatment. They were vaccinated against canine distemper, hepatitis and leptospirosis by the supplier. Animals were individually housed in suspended stainless steel cages and were maintained in a room that was kept at 70-76 degrees F and 25 - 50% relative humidity. The light cycle was 12 hr light/12 hours dark. Cages were cleaned semi-monthly.

Dogs were assigned to 4 test groups [0 mg/kg bw (control diet), or 7.5, 15 or 30 mg/kg bw test material] of 4 per sex per group one week prior to treatment by weight stratification. On day 1 of treatment, weights and ages of the animals were 5.1 - 6.7 kg and 4 - 5 months, respectively.

Test diets were prepared by geometrically diluting the appropriate amount of test material with Purina Laboratory Canine Diet in a grounded twin shell blender. Diets containing the least amount of test material were mixed first. The blender was completely cleaned between mixing each dose level. The formula used to determine the amount of material added to feed was as follows: mean body weight (g) x dose (mg/kg bw)/mean food consumption per day (g) = mg test material/kg diet. The desired doses were 7.5, 15.0 and 30.0 mg/kg bw. Doses were not adjusted for purity of the material. Doses were adjusted weekly based on the group body weights of dogs for that week. Diets were prepared weekly.

Animals were maintained on their respective diets for a period of 90 days. As mentioned above, food consumption and body weights were measured weekly. At study termination (days 90 and 9a), all animals were weighed.

They were then euthanized and exsanguinated. The gonads were weighed. Small portions of the following reproductive tissues were taken and preserved in 10% neutrally buffered formalin for possible histologic evaluation: ovary, prostate, testis, and uterus. Sections were prepared at 5 microns from paraffin blocks and stained with hematoxylin and eosin.

Data for organ weights were analyzed using a randomized block analysis of variance. The level of significance is $p < 0.05$. No statistical evaluations were performed on incidences of gross or microscopic lesions.

Test substance : The page describing the purity of the test material was missing from the report. However, the purity of the test material used in a rat study that was performed at the same facility around the same time was 90.0%. Impurities were not listed.

Reliability : (2) valid with restrictions
Doses of test material, homogeneous distribution, and stability in the diet were not confirmed analytically. The effects on mating and offspring were not tested.

14.04.2005 (20)

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

(1) Baldwin KL. 2002. CT-741-02. Ready biodegradability: CO2 evolution (modified Sturm Test). Toxicon Corporation Study Number 02J0014c for Cytec Industries Inc., dated July 23.

(2) Carpenter CP. 1973. Antioxidant A-1790, Range finding toxicity tests: single oral, single dermal, eye irritation (FHSA). Carnegie-Mellon University Report for American Cyanamid Company, dated September 17, 1973.

(3) Cytec Industries Inc. 2001. Material safety data sheet for Cyanox 1790 antioxidant, dated 5/29/2001.

(4) deVogel N. 2001. Chromosomal aberration assay with Cyanox 1790 antioxidant in cultured Chinese hamster ovary cells. TNO Nutrition and Food Research, Netherlands Organization for Applied Scientific Research Project 010.45007/01.02 for Cytec Industries BV, dated April 11, 2001.

(5) EPIWIN Aop (v1.90).

(6) EPIWIN BCF Program (v2.14)

(7) EPIWIN ECOSAR (v0.99).

(8) EPIWIN Fugacity Level III model program.

(9) EPIWIN Hydrowin (v1.67)

(10) EPIWIN Kowwin (v1.66).

(11) EPIWIN Mpbpwin (v1.40).

(12) EPIWIN Wskow (1.40).

(13) Glover S. 2002. CT-741-02. Acute toxicity to rainbow trout, Oncorhynchus mykiss, under static test conditions. Toxicon Corporation Study Number 02J0014d for Cytec Industries Inc., dated May 14.

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT